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PHYSIOLOGY OF THE WOOD-ROTTING BASIDIOMYCETES.[1] I. GROWTH AND NUTRITION IN SUBMERGED CULTURE IN SYNTHETIC MEDIA[2]

MARSHALL W. JENNISON, MELVA DERRICK NEWCOMB[3] AND RICHARD HENDERSON[4]

The growth requirements of only a small number of species of brown-rot and white-rot fungi have been studied in media of known composition (**6, 7, 8, 10, 11, 15, 18, 22, 25, 27, 28, 37**). Further, most previous studies of the wood-destroying and related Basidiomycetes have been carried out in stationary culture; a few recent investigations (**9, 10, 11, 22, 32**) have employed the known advantages (**5, 33**) of the submerged culture technique. At least quantitative and, perhaps, qualitative differences in growth and metabolic activity are to be expected between stationary and shaken cultures (**5**).

There is general agreement that, as regards vitamins, the majority of Hymenomycetes are partially or totally deficient only for thiamine (or its components); also, growth in malt extract, casein hydrolyzate and other complex organic mixtures generally is better than in a synthetic

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Syracuse University (NR 132-159). The studies were under the direction of M. W. Jennison.

[2] Portion of a thesis submitted by Melva Derrick to the Graduate School of Syracuse University for the Ph.D. in Microbiology.

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medium containing simple nitrogen compounds and supplemented with vitamins (**6, 7, 15, 16, 18, 20, 21, 25, 26, 27, 28, 37**). With certain wood-rot fungi growth has been obtained in synthetic media equal in amount to that in malt extract (**6, 15, 22, 25, 27, 28, 37**). For the few wood-rot fungi previously studied, both qualitative and quantitative differences have been found in the utilization of known organic and inorganic nitrogen compounds, including certain amino acids (**7, 8, 16, 18, 19, 20, 37**). Generally, ammonium nitrogen is assimilated (**6, 7, 8, 15, 16, 20, 26, 27, 28, 37**); a few wood-rot fungi utilize nitrate nitrogen slowly in stationary culture (**8, 16, 18, 20**). Our general knowledge of amino acid utilization has been augmented by the careful studies of Steinberg (**31**) with *Aspergillus niger*. This investigator correlated utilization of amino acids with molecular structure and believed that differences in utilization bore no relation to isomeric configuration. More recently, other workers (**13, 14, 24, 36**) have found, for a few species of molds and bacteria, that only the natural (L-) isomer of amino acids may be utilized. Increased growth of several thiamine-requiring fungi in inorganic nitrogen sources in the presence of certain organic acids has been demonstrated (**1, 8, 19**).

The present paper is a quantitative comparative study of the growth and nutrition of 42 species of brown-rot and white-rot fungi in submerged (shake) culture in synthetic media, with particular reference to utilization of known nitrogen compounds. The organisms used are representative of common wood-destroying forms.

MATERIALS AND METHODS

Most of the 42 cultures used (see TABLE II) were courteously furnished by Mr. Ross Davidson, U. S. Department of Agriculture; the others (numbers 1, 2, 4) were from Dr. Armando Russo, Instituto de Pesquisas Tecnológicas, São Paulo, Brazil. The stock cultures were maintained on potato dextrose agar (Difco, dehydrated).

All studies were carried out in shake culture, using 70 ml of medium in 250-ml Erlenmeyer flasks. In place of cotton plugs, which may introduce traces of extraneous nutrients into media, flasks were capped with a 4-inch square of special cellophane (Dupont, type 450 PT) which withstands autoclaving, held in place loosely by a rubber band. Also to minimize contamination of test media, special care was taken in cleaning glassware and in making media. Chemicals used were of the highest purity commercially obtainable. The inoculated flasks were incubated on a reciprocating shaking machine which had a stroke of 1.5 inches

and a speed of 110 three-inch cycles per minute. The standard time of incubation was 7 days unless otherwise specified. Experiment showed that the approximate optimum temperature for growth of most of the organisms was 28° C (± 1°); hence this temperature was used routinely for incubation.

In order to obtain reproducible quantitative results with the submerged culture technique, several conditions of procedure must be carefully controlled and standardized. The rate of growth depends upon the size and type of inoculum (**5**). A closer check among replicate cultures and a shorter lag period in initiation of growth are attained if the mycelial pellets (**2**) used as the inoculum are fragmented; the time of blending, washing and suspending of fragments, and amount of suspension used for the inoculum are important factors (**3**). The effect of inoculum in relation to the "carry-over" of nutrients—particularly vitamins—from one medium to another of different composition is a critical point (**12, 20**). Extensive preliminary experiments, not reported here, laid the foundation for fulfilling the above and other desiderata of technical procedure.

To produce the inoculum for test media, mycelium from stock cultures was transferred into a standard 1.0 percent malt extract (Difco, dehydrated) solution and grown for 7 days in shake culture. The resulting growth of mycelial pellets, after standardization, constituted the primary inoculum for each test medium; serial subcultures were then made in the test medium, each subculture being started with standardized growth from the preceding culture. The standardized growth used at each step in the inoculations was prepared as follows, using rigorous sterile precautions and controls for contamination. Mycelial pellets of a 7-day-old shake culture were fragmented for 1 minute in a sterile Waring blendor, the material in the resulting homogeneous suspension washed with sterile distilled water three times by centrifuging at 2000 rpm for 2 minutes, and resuspended in distilled water to 30 times the volume of packed fragments. The standard inoculum consisted of 0.15 ml (about 0.03 mg oven-dry weight) of such blended, washed mycelium per flask (70 ml) of medium.

Because of the "carry-over" of nutrients, by the inoculum, from one medium to another of different composition, growth in continued serial subculture must be obtained in a medium containing the test nutrient before it can be stated with certainty that the nutrient is utilized. In the studies reported here, the primary inoculum for each test medium consisted of cells from the standard 1.0 percent malt extract solution which supported growth of all the organisms. Serial transfers were then made

in a given test medium, using the standard inoculum procedure described above, until dry-weight determinations on successive 7-day-old subcultures showed that either the amount of growth produced had reached a constant level or that the organism had died out. Usually three serial transfers in a test nutrient resulted in a virtually constant amount of growth and, unless otherwise indicated, results are based on the third serial subculture.

All quantitative data on the value of individual test nutrients are based on the average dry weight of mycelium produced in the test media in duplicate flasks. The contents of each flask were filtered on an alundum crucible, the mycelial pellets washed, and dried to constant weight at 80° C in a vacuum oven.

In all of the nutrition studies a synthetic (chemically-defined) basal medium was used, to which each test nutrient was added singly. This basal medium, containing any one of several nitrogen sources, supported at least minimal growth of all but four of the organisms. The composition is shown in TABLE I.

TABLE I

BASAL SYNTHETIC MEDIUM

Glucose	10.0 gm
KH_2PO_4	1.5 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
Thiamine hydrochloride	1.0 mg
Nitrogen source	120 mg *nitrogen* (see below)
Trace elements (reference 2): B, 0.10 mg; Cu, 0.01 mg; Fe, 0.05 mg; Mn, 0.01 mg; Mo, 0.01 mg; Zn, 0.07 mg	
Distilled water	To make 1 liter

To this basal medium each nitrogen source (except malt extract) was added singly to give a concentration of 0.012 percent (120 mg/l) *total nitrogen*, except that amino acids which were available only as racemic mixtures (DL-) were used in a concentration of 0.024 percent total nitrogen, on the assumption—validated in the cases investigated (see Miscellaneous Studies, below)—that only the nitrogen of the natural (L-) form of the acid was utilizable. Also, ammonium nitrate was used in a concentration to give 0.012 percent *ammonium* nitrogen, because utilization of *nitrate* nitrogen could not be demonstrated. *It will be assumed* that all nitrogen sources used (except malt extract) were comparable on an equivalent "utilizable" nitrogen basis. The 1.0 percent malt extract solution contained approximately 0.004 percent total nitrogen.

All media were adjusted to give a pH of 5.0–5.5 after sterilization, except that the malt extract was used unadjusted (pH 4.9). This pH

range was found to be close to the optimum for most species under the conditions used. Most media or components were autoclaved at 121° C for 20 minutes; urea and heat-labile ammonium salts were Seitz-filtered. For the basal medium the inorganic salts, glucose, nitrogen source and thiamine were sterilized separately, then combined aseptically. Before inoculation media were cooled, then shaken for 1 hour on the shaking machine. For pH and Eh determinations (Beckman apparatus), at 22°–24° C, the shaken media were allowed to stand for 30 minutes. All Eh values are referred to the hydrogen electrode.

EXPERIMENTAL RESULTS

Growth and Acid Production in Shake Culture. The increase in cell substance with time, expressed as dry weight of mycelial pellets per 70 ml of culture medium, was determined for the 42 organisms in two media—1.0 percent malt extract solution and the basal synthetic medium (TABLE I) with L-glutamic acid as the nitrogen source. Enough replicate flasks were used to furnish samples periodically during the complete growth period, i.e., until the amount of growth reached a maximum. Determinations of the amount of mycelium (third, serial subculture) and of pH were made every two days, using the whole contents (70 ml) of duplicate flasks at each sampling period. Although the complete growth curves were determined in both media, only a portion of the data for malt extract are presented; similar results were obtained in the glutamic acid medium, with the differences noted below. TABLE II compares the mycelial weights after 7 days (the standard growth time for the nutrition studies, below) with the maximum amount of growth attained and the time required to reach this maximum. Also, the maximum acidities attained, expressed as pH, are shown.

In TABLE II differences among organisms in rate of growth are evident from the 7-day mycelial weights. *P. radiculosa* and *A. mellea,* and to a lesser extent *M. americanus* and *P. sulphureus,* were notably slow-growing. Plots of the growth curves demonstrated that in no case did the rate of growth decrease markedly before the 8th day, either in the malt extract or in the synthetic medium. Comparison of the maximum mycelial weights with the 7-day data (TABLE II) clearly shows, however, that the growth of most species leveled off sharply—i.e., was virtually complete (80–90 percent)—at the end of 7 days. These results indicate that in general 7-day growth was a good relative measure of total growth; *P. radiculosa* was the outstanding exception.

Acid was invariably produced in the malt extract solution, and maxi-

TABLE II

SEVEN-DAY GROWTH, MAXIMUM GROWTH AND MAXIMUM ACIDITY (AS pH) OF WOOD-ROT FUNGI IN ONE PERCENT MALT EXTRACT, INITIAL pH 4.9. DRY MYCELIAL WEIGHT PER 70 ML CULTURE

Organism	Original culture number	Seven-day mycelial weight, mg	Maximum mycelial weight		Maximum acidity, as pH	
			Mg	Days	pH	Days
Brown rots						
Coniophora puteana (cerebella)	2	79	132	16	3.0	12
Daedalea quercina	FP 57076-S	113	126	12	2.0	14
Fomes meliae	FP 50336-R	126	125	8	2.2	20
F. officinalis	F 1276	63	71	14	2.3	14
F. subroseus	Snell 11	106	116	10	2.3	14
F. subroseus	Snell 20	99	104	10	4.1	10
Hydnum pulcherrimum	FP 81027-R	82	92	14	2.4	16
Lentinus lepideus	Mad. 534	66	80	18	1.6	20
Lenzites saepiaria	Mad. 537	83	94	10	4.3	12
L. striata	1	65	86	12	3.2	20
L. trabea	Mad. 539	54	71	14	3.5	20
Merulius americanus	FP 94365	30	59	40	2.8	8
Polyporus betulinus	FP 58514-S	80	91	12	2.4	16
P. immitus	FP 71384	88	95	10	2.5	8
P. palustris	FP 94152	110	114	8	1.7	12
P. schweinitzii	FP 71356-S	57	75	16	4.2	20
P. spraguei	FP 14857-S	115	132	10	3.0	12
P. sulphureus	FP 48603-S	30	52	18	3.0	20
Poria cocos	FP 71051	48	74	16	2.8	18
P. incrassata	Mad. 563	80	93	12	3.0	6
P. radiculosa	FP 94373-R	12	43	40	1.5	20
P. monticola	Mad. 575	116	131	12	2.1	6
P. nigra	FP 71118	147	155	10	2.3	16
P. oleraceae	FP 48282-R	123	138	10	2.2	10
P. vaillantii	FP 90877-R	96	120	14	1.8	18
P. xantha	FP 192-Sporo.	72	90	12	3.5	10
Ptychogaster rubescens	UIFP 716	89	102	14	3.7	16
Trametes malicola	FP 71956	65	70	8	1.7	18
T. serialis	FP 11977	121	140	10	4.5	10
Brown rots—av.		86.4	102.1	14.1	2.7*	14.3
White rots						
Armillaria mellea	FP 46700	11	22	20	4.8	16
Fomes annosus	FP 90898-R	94	122	20	3.5	20
F. fomentarius	FP 59009-S	71	85	10	3.5	8
F. geotropus	FP 55521-S	123	161	12	4.3	8
F. pini	FP 71757	60	92	14	4.0	12
Lentinus tigrinus	Mad. 466	100	121	14	4.3	14
Peniophora gigantea	FP 56475-S	97	117	14	2.8	18
Polyporus abietinus	FP 71429-R	153	277	20	3.9	6
P. anceps	FP 58526-R	102	114	8	2.6	8
P. fumosus	4	64	84	12	1.5	10
P. tulipiferus	Mad. 517	130	137	10	3.9	4
P. versicolor	FP 57034-R	118	151	10	3.7	6
P. subacida	FP 71955	97	115	12	3.5	12
White rots—av.		93.8	123.0	13.5	3.6*	10.9

* Average of actual pH values, not of H-ion concentrations.

mum acidity (as pH) was attained in two weeks or less: by the white-rot fungi in 10.9 days and by the brown-rot species in 14.3 days. The lowest pH values (1.5–1.7) were produced by *P. palustris, P. fumosus, L. lepideus, P. radiculosa* and *T. malicola.* With most organisms the *terminal* pH (at the time of maximum mycelial weight) was virtually the same as the *maximum* pH recorded, that is, there was little evidence for the reversal of the reaction, with age, from acid towards alkaline. As between the white-rot fungi and the brown rots, the latter group averaged a little less growth and produced a slightly lower average pH than did the white-rot organisms.

It was mentioned above that growth of the 42 organisms was determined both in the malt extract and in the basal synthetic medium (TABLE I) with glutamic acid as the nitrogen source. Growth data for the latter medium are not presented because of many similarities to the results for malt extract. On the average, the glutamic acid medium supported about three-quarters as much growth as did the malt extract; a slightly lower average pH was reached in the latter medium. The most significant difference in growth-supporting ability of the two media was that four of the organisms—*F. officinalis, P. radiculosa, P. vaillantii* and *A. mellea*—did not grow in continued subculture in the synthetic medium under the *standard* conditions used. The reasons for this will be considered in the section on Special Growth Conditions.

Utilization of known forms of organic and inorganic nitrogen in synthetic media under "standard" conditions. These studies were carried out in shake culture with the basal medium and other conditions previously described, viz., standard inoculum; initial pH of media 5.0–5.5; total nitrogen concentration in basal medium of 0.012 percent, except for ammonium nitrate and DL- mixtures of amino acids, 0.024 percent; and 7-day incubation. A mixture of amino acids, as vitamin-free casein hydrolyzate (General Biochemicals), was used as a basis for comparison of growth in single nitrogen compounds. Fifteen representative organisms of the 42 in TABLE II were used. Growth was determined quantitatively in the basal medium (TABLE I) containing one of each of the following nitrogen sources: casein hydrolyzate, twenty-five single amino acids and amides (General Biochemicals), urea, ammonium carbonate, ammonium sulfate, ammonium nitrate, ammonium chloride, potassium nitrate and potassium nitrite (Baker's analyzed). All tests were run both with and without thiamine (1 mg/l). With the exception of two organisms, *P. rubescens* and *T. serialis,* growth did not take place in the synthetic media in the absence of thiamine, and the summarized data (TABLE III) show only the results *with* thiamine. All individual data

are averages of duplicate sample flasks. To furnish a basis for statistical evaluation of the replication of results over the period of months required to study all of the organisms and nutrients, control cultures of each organism in 1.0 percent malt extract solution were quantitated concurrently with each test nitrogen source.

As indicated in the section on Materials and Methods, serial subcultures in a given test nutrient must be carried out until growth either reaches a constant level (after a given incubation time) or dies out. The original growth data are shown in TABLE III, below: under each nitrogen source the figures in the first column refer to the amounts of growth obtained in the third, serial, 7-day-old subculture; the figures, if any, in the second column are for the ninth, serial, 7-day-old subculture. If an organism produced in the third transfer in a single nitrogen source less than about 50 percent as much growth as in the casein hydrolyzate, the organism was carried through six more serial subcultures in order to make certain that growth did or did not continue; in some cases, as a check, organisms were serially transferred a total of nine times regardless of the amount of growth in the third transfer. Only third-transfer data were used for analysis of results.

Since with most organisms growth was only *virtually* complete in 7 days (TABLE II), this incubation period actually gave data which were indicative of relative rates of utilization of a compound during the period of maximum growth-increase rather than showing absolute utilizability.

With a single exception (*T. serialis* in ammonium chloride) none of the organisms grew in the standard synthetic medium containing ammonium chloride, potassium nitrate or potassium nitrite after several (3–7) serial subcultures. These nitrogen sources are therefore omitted from TABLE III. Growth of *T. serialis* in ammonium chloride, and the special conditions for the utilization of this nitrogen source by the other organisms, will be considered later.

In order to determine whether the differences in the utilization of various nitrogen compounds were significant, as regards both organisms and nutrients, the data in TABLE III, below, were treated statistically. The two sources of experimental error (i.e., variation due to chance) to be evaluated were: (a) the error of the mean of duplicate samples run at the same time, and (b) the error of replication of samples run at different times over a period of months. After evaluating these errors (average variance of 2.6 mg and 12.8 mg, respectively) the data were analyzed by the method given by Tukey (**34**) for comparing and separating individual means in the analysis of variance. The problem, as stated by him, is that, "We wish to separate the varieties [nitrogen

sources] into distinguishable groups as often as we can without too frequently separating varieties [nitrogen sources] which should stay together." Separation of the nitrogen sources into groups as regards statistically-significant (0.05 level) differences in growth-supporting ability, both for each organism individually and for all organisms together, is discussed following TABLE III. For individual organisms, and for the brown rots and white rots separately, division of the nitrogen sources into statistically-significant groups is, of course, less reliable than for all organisms together.

TABLE III summarizes test data on the growth of 15 brown-rot and white-rot fungi with single organic and inorganic nitrogen compounds, compared with casein hydrolyzate, in the basal medium. The amino acids and amides are grouped together, as are the ammonium salts and urea, with the compounds in alphabetical order.

Considering first the *organic* nitrogen compounds, it is seen from TABLE III that except for L-cysteine, under the *standard* conditions used each of the amino acids and amides singly in the basal medium was utilized by all organisms in continued subculture. Large quantitative differences are evident both among organisms and among nitrogen sources as regards amounts of growth produced. Except for L-cysteine, the Eh values (referred to the hydrogen electrode) of the media ranged from about + 400 millivolts to + 500 mv, with the majority in the range of + 450 to + 500 mv. The L-cysteine medium had a substantially lower Eh—about + 350 mv. This amino acid, often used in bacteriological culture media to bring about reduced conditions for the growth of anaerobes, apparently produced an O-R potential too low for growth of the "aerobic" wood rots. When ascorbic acid (ca. 1.5 gm/l) was added to the cysteine medium (0.012 percent nitrogen) to raise the Eh to about + 450 mv, other conditions being the same as previously used, continued growth of all organisms was obtained (TABLE III). The growth obtained in the oxidized [5] cysteine medium was comparable in amount to that produced by the respective organisms in several other amino acid media ("standard" Eh of + 400 to + 500 mv) and averaged, for the 15 species, 43 percent of that obtained in casein hydrolyzate. In the cysteine medium without ascorbic acid, continued growth of several organisms also was obtained by decreasing the concentration of cysteine

[5] Although ascorbic acid has reducing properties, in aqueous solution it may undergo auto-oxidation to dehydroascorbic acid and hydrogen peroxide (**4**). Also, the O-R potential of the ascorbic/dehydroascorbic system is higher than that of cysteine/cystine (**17**). The net effect of the addition of ascorbic acid to the cysteine medium was to raise the potential of the latter.

TABLE III

GROWTH OF CERTAIN WOOD-ROT FUNGI IN BASAL SYNTHETIC MEDIUM WITH VARIOUS ORGANIC AND INORGANIC NITROGEN SOURCES. UNDER EACH NITROGEN SOURCE, FIGURES IN THE FIRST COLUMN ARE THIRD-TRANSFER DATA; IN THE SECOND COLUMN, NINTH SERIAL TRANSFER

Organism \ Nitrogen source	Casein hydrolyzate	DL-alpha alanine	Beta alanine	L-arginine HCl	L-asparagine	DL-aspartic acid	L-cysteine* HCl	L-cystine
	Milligrams of mycelium per 70 ml culture							
Brown rots								
Daedalea quercina	110	23; 29	51	22; 29	23; 30	31; 25	0(33; 31)	57
Fomes subroseus (Snell 20)	81	80	35; 29	73	71	72	0(47)	42; 36
Hydnum pulcherrimum	69	45	24; 31	62	62	72	0(33; 38)	32; 27
Lentinus lepideus	109	46; 44	37; 37	30; 21	41	33; 24	0(13; 18)	12; 9
Lenzites trabea	44; 47	87	29; 33	88	87	90	0(41)	35
Polyporus palustris	59	78	48; 40	81	90	82	0(56)	51
Poria monticola	93	76	26; 27	83	87	86	0(25; 27)	20; 25
*Ptychogaster rubescens***	133	50	16; 33	97	95	114	0(29; 37)	6; 11
*Trametes serialis***	120	60	13; 20	50; 47	49; 44	38; 30	0(43; 50)	20; 16
Brown rots—av.	**91**	**61**	**31**	**65**	**67**	**69**	**0(36)**	**31**
White rots								
Fomes annosus	75	59	5; 7	68	68	67	0(13; 15)	3; 6
Lentinus tigrinus	103	91	40; 35	92	92	93	0(47; 38)	58
Peniophora gigantea	141	96	13; 19	113	110	115	0(23; 29)	14; 9
Polyporus anceps	89	70	31; 26	67	62	68	0(46; 45)	68
P. tulipiferus	60	96	20; 25	83	86	86	0(60)	32; 38
Poria subacida	55	42	20; 26	55	49	51	0(46)	29; 21
White rots—av.	**87**	**76**	**21**	**80**	**78**	**80**	**0(39)**	**34**
All organisms—av.	**89**	**69**	**26**	**73**	**73**	**75**	**0(38)**	**33**

TABLE III—*Continued*

Organism \ Nitrogen source	L-glutamic acid	Glutamine	Glycine	L-histidine HCl	L-hydroxy-proline	DL-iso-leucine	L-leucine	L-lysine HCl
	Milligrams of mycelium per 70 ml culture							
Brown rots								
Daedalea quercina	38; 38	26; 30	21; 27	35; 40	31; 26	29; 21	19; 24	25; 30
Fomes subroseus (Snell 20)	74	80; 84	72	42; 34	59; 51	40; 37	49	35; 38
Hydnum pulcherrimum	34; 31	24; 29	55	31; 33	41; 45	42	45	19; 23
Lentinus lepideus	21; 25	41; 47	29; 31	11; 10	34; 31	10; 20	12; 20	15; 19
Lenzites trabea	90	91	89	51	85	60	69	31
Polyporus palustris	90	91	80	39; 44	80	52; 47	51; 55	31; 26
Poria monticola	85	90; 103	83	51; 40	64; 56	36; 32	55; 51	32; 35
*Ptychogaster rubescens***	119	118	105	52	104	89	102	30; 38
*Trametes serialis***	62; 63	60; 65	50; 54	30; 38	40; 46	31; 40	53; 54	25; 28
Brown rots—av.	**68**	**69**	**65**	**38**	**60**	**43**	**51**	**27**
White rots								
Fomes annosus	70	71	65	33; 26	63	42; 37	42	28; 30
Lentinus tigrinus	100	112	90	50; 72	50; 39	57	70	34; 34
Peniophora gigantea	115	134	112	49; 53	72	73	81	22; 27
Polyporus anceps	61	66	64	29; 37	47; 40	36; 30	45; 37	19; 15
P. tulipiferus	90	102	79	46; 55	41; 35	47; 41	58; 47	28; 28
Poria subacida	52	72	49	20; 33	61	30; 26	30; 24	19; 27
White rots—av.	**81**	**93**	**77**	**38**	**56**	**48**	**54**	**25**
All organisms—av.	**75**	**81**	**71**	**38**	**58**	**46**	**53**	**26**

TABLE III—*Continued*

Organism \ Nitrogen source	DL-methionine	DL-norleucine	DL-ornithine HCl	DL-phenylalanine	L-proline	DL-serine	DL-threonine	L-tryptophane
	Milligrams of mycelium per 70 ml culture							
Brown rots								
Daedalea quercina	22; 32	33; 22	20; 26	35; 32	24; 23	26; 29	24; 26	20; 24
Fomes subroseus (Snell 20)	42; 28	40; 47	68	51	70	53	55	56
Hydnum pulcherrimum	45	22; 28	58	46	58	47	49	48
Lentinus lepideus	12; 11	16; 12	17; 24	10; 16	32; 29	20; 14	16; 17	15; 19
Lenzites trabea	11; 6	48	82	65	84	72	73	73
Polyporus palustris	63	47; 65	80	57; 50	83	66	68	67
Poria monticola	60	44; 30	81	54; 59	82	63	63	64
*Ptychogaster rubescens***	100	50	110	96	104	101	87	97
*Trametes serialis***	36; 50	21; 30	31; 40	37; 26	48; 57	39; 43	37; 30	35; 28
Brown rots—av.	**43**	**36**	**61**	**50**	**65**	**54**	**52**	**53**
White rots								
Fomes annosus	37; 33	30; 40	59	41; 37	61	44	43	43
Lentinus tigrinus	59	47; 41	84	65	90	74	77	78
Peniophora gigantea	87	44; 41	103	81	110	88	89	90
Polyporus anceps	47; 37	36; 39	60	41; 34	64	49	48	50
P. tulipiferus	61; 77	34; 40	79	54; 60	84	68	66	65
Poria subacida	35; 27	25; 19	45	25; 29	46	24; 28	35; 31	34; 20
White rots—av.	**54**	**36**	**72**	**51**	**76**	**58**	**60**	**60**
All organisms—av.	**49**	**36**	**67**	**51**	**71**	**56**	**56**	**57**

TABLE III—*Continued*

Organism \ Nitrogen source	L-tyrosine	DL-valine	$(NH_4)_2CO_3$	NH_4NO_3	$(NH_4)_2SO_4$	Urea
	Milligrams of mycelium per 70 ml culture					
Brown rots						
Daedalea quercina	30; 25	90	57; 49	67; 59	41; 43	38; 55
Fomes subroseus (Snell 20)	40; 31	86	50; 43	50; 42	38; 37	22; 53
Hydnum pulcherrimum	29; 26	40	12; 19	25; 28	10; 21	25; 23
Lentinus lepideus	12; 8	45; 47	59; 46	74; 78	48; 59	0
Lenzites trabea	53	104	23; 21	25; 17	13; 14	16; 54
Polyporus palustris	42; 45	97	52; 48	47; 50	47; 52	28; 54
Poria monticola	34; 41	75	37; 29	37; 36	30; 31	35; 30
*Ptychogaster rubescens***	50; 60	192	87; 65	86; 70	64; 72	36; 31
*Trametes serialis***	29; 23	68; 60	47; 45	53; 48	39; 36	66; 67
Brown rots—av.	**35**	**89**	**47; 40**	**52; 48**	**37; 41**	**30; 41**
White rots						
Fomes annosus	30; 39	102	5; 11	59; 48	5; 7	8; 20
Lentinus tigrinus	54	101	22; 20	32; 32	13; 16	11; 25
Peniophora gigantea	50; 43	98	45; 39	47; 43	33; 28	35; 46
Polyporus anceps	20; 31	71	31; 27	47; 42	29; 21	15; 36
P. tulipiferus	36; 38	94	58; 53	64; 61	43; 49	26; 61
Poria subacida	18; 22	52	54; 49	53; 50	43; 40	17; 16
White rots—av.	**35**	**86**	**36; 33**	**50; 46**	**28; 27**	**19; 31**
All organisms—av.	**35**	**88**	**42; 37**	**51; 47**	**33; 34**	**26; 36**

* No growth in this medium at its "standard" Eh of + 350 mv. Figures in parentheses show third-transfer and ninth-transfer data, respectively, in modified cysteine medium, Eh = + 450 mv (see text).

** Will grow without thiamine in all media shown.

to 0.006 percent nitrogen, resulting in a somewhat less reduced medium (ca. + 400 mv) than with the standard higher cysteine concentration. It is apparent that L-cysteine was a nutritionally-utilizable form of nitrogen under an environmental condition comparable to that under which the other amino acids were assimilated, i.e., a more highly oxidized medium.

Only *T. serialis* and *P. rubescens* grew in continued subculture in each of the amino acids *without* thiamine, although the amount of growth after 7 days usually was less than *with* thiamine.

Statistical analysis by Tukey's method of the data in TABLE III separated the amino acids and amides as follows as regards average growth-supporting ability for all organisms together, *expressed as percent of growth in casein hydrolyzate:* casein hydrolyzate (100 percent), DL-valine (99), glutamine (91) > DL-aspartic acid (84), L-glutamic acid (84), L-arginine (82), L-asparagine (82), glycine (80), L-proline (80), DL-alpha alanine (78), DL-ornithine (75) > L-hydroxyproline (65), L-tryptophane (64), DL-serine (63), DL-threonine (63), L-leucine (60), DL-phenylalanine (57), DL-methionine (55), DL-isoleucine (52) > L-histidine (43), DL-norleucine (40), L-tyrosine (39), L-cystine (37) > beta alanine (29), L-lysine (29), L-cysteine (0–43, see text above). Because a possible effect of isomerism—utilization of L-isomers and non-assimilation of the D-form of amino acids—was taken into account experimentally, the above grouping presumably results primarily from differences in molecular structure other than isomeric configuration. Casein hydrolyzate, valine and glutamine are clearly separable from all of the other nutrients, on an equivalent "utilizable" nitrogen basis. Its complement of several amino acids notwithstanding, casein hydrolyzate was not a significantly better nutrient than the single amino acid valine or the amide of glutamic acid, glutamine. The difference in growth-supporting ability between the aspartic acid-ornithine group of nitrogen compounds and the hydroxyproline-isoleucine group was statistically significant. Histidine, norleucine, tyrosine and cystine formed a group nutritionally better than beta alanine and lysine, and superior to cysteine under the *standard* experimental conditions used. Whether cysteine should be placed in the histidine-cystine group is less important than the fact that it was nutritionally-utilizable under proper environmental conditions.

On the average there was some correlation, on an equivalent "utilizable" nitrogen basis, between the amount of growth produced and the type or structure—other than isomerism—of single amino acids, although this was not consistently the case with individual organisms.

Of the aliphatic, monoamino, monocarboxy acids containing no additional functional groups, those of shorter (3–4 carbon) chain length—glycine, valine, alpha alanine—averaged significantly more growth (78–99 percent of that in casein hydrolyzate) than the 5- or 6-carbon acids leucine, isoleucine and norleucine (40–60 percent). The short-chain acids containing an OH group—serine and threonine—were considerably poorer nutrients (63 percent of the growth in casein hydrolyzate) than glycine, valine and alpha alanine; those containing sulfur—cystine, cysteine, methionine—were even more poorly assimilated, although methionine (55 percent) was equal to or better than several non-sulfur-containing amino acids. The monoamino, dicarboxy acids—aspartic, 4-carbon; glutamic, 5-carbon—and their corresponding amides (diamino), glutamine and asaparagine, were equally as good (82–91 percent) as glycine, valine and alpha alanine. Two of the diamino, monocarboxy acids—the 5-carbon-chain arginine and ornithine—were utilized to the same extent (75–82 percent) as glycine (80 percent); the 6-carbon lysine was low (29 percent) and consistently poor. The aromatic amino acids, phenylalanine and tyrosine, were similar in growth-supporting ability (39–57 percent) to the sulfur-containing aliphatic compounds. The heterocyclic tryptophane, histidine, proline and hydroxyproline fitted into no particular pattern: proline was a good nitrogen source (80 percent relative utilization) and histidine (diamino) a poor one (43 percent), with the others intermediate (64–65 percent) in growth-supporting ability. Compared with alpha alanine (78 percent of the growth in casein hydrolyzate), beta alanine supported much less growth on the average (29 percent); the amino group in the beta-position appeared to have a profound effect on utilizability.

As between the brown-rot fungi and the white-rot species, calculation of the rank-difference correlation coefficient (**35**) for average utilization of the various amino acids gave a value of 0.981. This indicates virtually no significant difference, on the average, between these two groups in the assimilation of the several nitrogen sources.

As a matter of interest rather than of strict comparison on the basis of known nutrients, the difference may be noted in average amount of growth for the 15 organisms in casein hydrolyzate and in malt extract. The 7-day mycelial weights in casein hydrolyzate averaged 89 mg (TABLE III) and for the same species in malt extract averaged 98 mg (TABLE II). By the Tukey analysis this difference is not statistically significant.

For *individual* organisms the Tukey analysis showed that the organic nitrogen sources fell into graded series with several quantitatively-

significant breaks, although these breaks often were not identical with those for all organisms together. Casein hydrolyzate was significantly better than any single nitrogen compound for 5 organisms out of the 15—*D. quercina, H. pulcherrimum, L. lepideus, T. serialis* and *P. anceps.* Valine was best in 4 cases—for *L. trabea, P. palustris, P. rubescens* and *F. annosus,* and glutamine in one case—for *P. subacida.* Equally with casein, glutamine was significantly the best for *P. gigantea.* For the 4 remaining organisms, casein, valine or glutamine was best, but not significantly so. Generally, the best utilized of the single organic nitrogen compounds were valine, glutamine, glutamic acid, aspartic acid, asparagine and arginine. Lysine and beta alanine were fairly consistently, although not invariably, the poorest of the nitrogen compounds which supported growth.

Certain outstanding differences between the *average* utilization of amino acids and utilization by individual organisms are worthy of mention. For the majority of the organisms cystine supported relatively little growth, but *D. quercina* utilized it better than any single amino acid except valine; for *P. anceps* cystine was a better nitrogen source than most other amino acids. Similarly, beta alanine, although usually at the bottom of the list, supported more growth of *D. quercina* and *L. lepideus* than did the majority of single amino acids. Glycine and glutamine, in most cases readily utilized, were poor for *D. quercina; H. pulcherrimum* also produced small amounts of mycelium in the latter. *D. quercina* was outstanding as regards the number of amino acids in which the amount of growth it produced was quantitatively either much greater or much less than the average for all organisms. *L. trabea* produced as much or more mycelium in a majority of the single amino acids than in casein hydrolyzate. Markedly less growth was produced in a larger proportion of the single amino acids by *D. quercina, L. lepideus* and *T. serialis* than by the other organisms. *L. lepideus* produced more mycelium in each of the inorganic nitrogen sources than it did in any single amino acid. Some of these apparent differences among species as regards the quantitative utilization of specific nitrogen compounds presumably are real differences, while to some extent they may also be reflections of dissimilar growth rates of the organisms.

Considering TABLE III as regards the *inorganic* nitrogen sources and urea, there were large quantitative differences among organisms in the amounts of growth produced, although on the average the differences in utilization of these various nitrogen sources were not great. For both the brown-rot fungi as a group and for the white-rot species, there was

a consistent decrease in average growth-supporting ability from ammonium nitrate to urea. In urea there was substantial increase in the average amount of growth from the third to the ninth serial subculture, with both the brown rots and the white rots. Presumably this increase reflected some degree of adaptation of certain organisms to the nutrient. *M. americanus* showed a marked increase in growth in casein hydrolyzate and in ammonium sulfate, and *F. fomentarius* in ammonium carbonate, from the third to the ninth serial transfer. Several species were outstanding in this respect in urea.

As mentioned above, the negative data for growth in ammonium chloride, potassium nitrate and potassium nitrite were not included in TABLE III, since *T. serialis* was the only culture which could utilize even one of these compounds under the conditions used. This organism produced in the ammonium chloride medium, in the third subculture, 44 mg of growth, and 40 mg in the ninth serial transfer. (Utilization of ammonium chloride by the other organisms under certain conditions is considered in the section on Special Growth Conditions, below.)

Based upon average growth-supporting ability, *expressed as percent of growth in casein hydrolyzate,* statistical analysis by Tukey's method showed that the inorganic nitrogen compounds and urea in TABLE III should be grouped as follows: casein hydrolyzate (100 percent) > ammonium nitrate (57), ammonium carbonate (47), ammonium sulfate (37), urea (29). Clearly, the casein hydrolyzate was far superior to the single nitrogen compounds; the latter are more closely grouped. That growth in ammonium nitrate (used at 0.024 percent total nitrogen concentration) was not significantly greater than in ammonium carbonate (at 0.012 percent nitrogen) suggests no appreciable utilization of nitrate nitrogen and confirms the assumption that the inorganic nitrogen compounds are comparable on an equivalent "utilizable" nitrogen basis. Apparent differences in utilization of the various ammonium compounds may result in part, at least, from differences in hydrogen ion concentration produced in the media. It may be noted that the Tukey analysis is applicable only to *adjacent* means and groups; it does not show, for example, whether the difference in utilization between ammonium nitrate and urea, above, is significant. As between the brown-rot fungi as a group and the white-rot species, the order of average utilization of the nitrogen sources was identical for these groups considered separately and together, and there was no statistically-significant difference in the average amount of growth produced by each group in the same nitrogen source. Apparently the brown-rot fungi and the

white rots did not differ quantitatively, on the average, on the above nutritional basis.

Based on growth-supporting ability for *individual* organisms, statistical analysis of the data on ammonium salts and urea showed with most organisms a fairly consistent decrease in nutritive value from ammonium nitrate to urea, but often without any significant breaks. A few individual species of fungi showed marked deviations from the average in utilization of certain of these compounds. *P. rubescens* grew especially well in all of the inorganic nitrogen sources; *H. pulcherrimum, L. trabea* and *L. tigrinus* grew rather poorly. *F. annosus* produced appreciable growth only in ammonium nitrate. For 9 organisms out of 15 used, urea supported the least amount of growth; ammonium sulfate was poorest in 5 cases and, equally with ammonium carbonate, was least utilized in another case. Ammonium nitrate alone, or equally with ammonium carbonate in 5 cases, was the best of the inorganic nitrogen compounds for 14 of the 15 organisms, but significantly so in only 5 instances. For the other organism, *T. serialis,* urea supported significantly the greatest amount of mycelium.

Although the data on growth in ammonium salts and urea for only 15 organisms are presented in TABLE III, growth of the remainder of the 42 species in TABLE II also was determined in these nitrogen sources. All of the organisms except *F. officinalis, P. radiculosa, P. vaillantii* and *A. mellea*—which did not grow in the standard synthetic medium with glutamic acid—grew in continued serial subculture in the basal medium with each inorganic nitrogen compound or urea.

Special (non-standard) growth conditions of certain fungi. In relation to TABLE II, it was noted that four organisms—*F. officinalis, P. vaillantii, P. radiculosa* and *A. mellea*—which grew in the malt extract did not grow in continued subculture in the synthetic medium with L-glutamic acid. Also, the latter two species, and *M. americanus* and *P. sulphureus,* produced relatively little mycelium even in malt extract. The special growth requirements of these organisms were therefore investigated. Further, the lack of growth in ammonium chloride of all organisms except *T. serialis* (see discussion of TABLE III) invited clarification.

For *P. radiculosa* it was determined that the poor growth in malt extract and the lack of measurable growth in the synthetic medium were due to inhibition by acid production. Daily pH readings in inoculated malt extract and in synthetic media showed that the pH dropped very rapidly, acid being produced as the result of only a few milligrams of growth (TABLE II). By readjusting the pH at 2-day intervals during

incubation, growth of *P. radiculosa* in amount comparable to that of other organisms was obtained in serial subculture in the basal medium with a variety of nitrogen sources; for example, 50 mg with glutamic acid. Thus, what at first appeared to be an inability of this organism to utilize certain nitrogen compounds was merely a reflection of special environmental requirements resulting from a marked sensitivity to hydrogen ion concentration.

The poor growth of *M. americanus* was found to be due to the standard temperature of incubation used (28° C), which was about 4° C above the optimum for this organism. Growth at 24° C was found to be of the same order of magnitude as that of other organisms.

It was determined that optimum conditions of temperature (26° C) and pH (5.0–5.5) for *P. sulphureus* did not result in any appreciable increase in growth, and that poor growth was not due to acid production. Neither did changes in the Eh of media effect greater yields of mycelium. Under the environmental conditions used and investigated, this organism apparently was "naturally" a slow-growing species, i.e., no reason for its poor growth could be found.

The poor growth of *A. mellea* in malt extract and its original inability to grow in the glutamic acid medium apparently were due both to the degree of oxidation of the milieu and, in the synthetic medium, to an unsuitable quantitative balance of medium components. As regards the effect of O-R potential, it appeared that this organism required a more reduced condition than that of most of the media (Eh = + 400 to + 500 mv). Relatively good growth of *A. mellea* was obtained in the basal medium with L-cysteine (in which the other organisms did not grow under our "standard" conditions) at 0.012 percent cysteine nitrogen concentration, Eh = + 350 mv, and at 0.024 percent nitrogen concentration, Eh = + 290 mv. Also, growth comparable to that of some of the other fungi in more highly oxidized media was obtained with *A. mellea* in malt extract, in casein hydrolyzate and in glutamic acid when these media were reduced to an initial Eh of + 325 mv with either cysteine or sodium thioglycollate. Larger amounts of growth resulted when a nitrogen atmosphere was used in addition to one of the reducing agents. It seems clear that poor growth was not due to a nutritional deficiency per se, but in part to the degree of oxidation of the media. Of the 42 organisms studied, *A. mellea* was unique in its low Eh requirements. The other factor in addition to Eh which affected the growth of *A. mellea* in the standard glutamic acid medium was the quantitative composition of the latter. Although the organism did not grow in this medium originally, it was found possible to adapt *A. mellea*

to grow and produce measurable amounts (10–20 mg) of mycelium therein, at an unadjusted Eh of about + 400 mv, by continued serial subculture in the medium. Later, a quantitatively more nearly optimal synthetic medium was developed, in which this organism grew profusely without Eh adjustment (see next section).

As regards the lack of growth of *P. vaillantii* and *F. officinalis* in the synthetic medium, certain essential nutrients were shown to be lack-

TABLE IV

SYNTHETIC MEDIA FOR WOOD-ROTTING FUNGI NOT GROWING IN "STANDARD" SYNTHETIC MEDIUM

Constituent	A Original basal medium (TABLE I)	B Composition of new media for growth of indicated fungi. Growth data are for cultures 7 days old	
		F. officinalis	*P. vaillantii*
Glutamic acid*	0.012	0.10	0.06
Glucose*	1.00	10.0	10.0
KH_2PO_4*	0.15	0.7	0.5
$MgSO_4 \cdot 7H_2O$*	0.05	0.03	0.05
Thiamine**	1.00	2.0	3.0
Trace elements**— B, 0.10; Cu, 0.01; Fe, 0.05; Mn, 0.01; Mo, 0.01; Zn, 0.07		Same as original basal medium (A)	
Adenine**	0	18.0	20.0
Additional requirements		0	biotin, 2 μg/l; riboflavin, 2 mg/l
Initial pH	5.0–5.5	4.5	5.0
		Growth in media	
Growth in malt extract,† mg		63	96
Growth in B, mg		296	115
Growth increase (B/malt extract × 100), percent		470	120

* Expressed in percent. For glutamic acid, as *percent nitrogen*.

** Expressed as mg/l of medium.

† No growth in original basal medium (A), so malt extract (pH 4.9) used for comparison.

ing in the standard basal medium. Both organisms were found to require adenine in addition to thiamine, and *P. vaillantii* also required biotin and riboflavin for growth. Determination of the approximately optimal levels of these nutrilites, together with quantitative changes in the constituents of the standard basal medium, resulted in new media which supported good growth of the organisms. Quantitative changes in the standard basal medium were made in a manner similar to that

used in developing "optimal" synthetic media for certain organisms (see next section). TABLE IV compares the composition of the standard and the new synthetic media; the percentage growth-increase in the latter is compared with malt extract, as there was no growth of the organisms in the original basal medium.

Nitrogen compounds other than glutamic acid were not tried in the new synthetic media (TABLE IV), but it is probable that other nitrogen sources would support growth in the presence of the additional nutrilites.

With regard to lack of growth in the ammonium chloride medium of any organism except *T. serialis*, it was found that some of the cultures utilized this compound in the presence of succinic acid. Other organic acids were not tried. Further investigation showed that at least the following organisms would grow in continued serial subculture in the basal medium plus ammonium chloride (at 0.012 percent nitrogen) plus succinic acid (10 mg/l): *P. radiculosa, P. palustris, L. trabea, P. monticola, L. tigrinus, P. anceps, P. rubescens, P. gigantea, H. pulcherrimum, L. lepideus* and *P. subacida*. Also, the addition of succinic acid to media containing the other ammonium compounds (ammonium nitrate, ammonium carbonate, ammonium sulfate) markedly stimulated growth of most of the fungi. The greatest stimulation was with *F. annosus*, the growth of which in ammonium carbonate was increased 10-fold with succinic acid (cf. TABLE III). *T. serialis*, which utilized ammonium chloride in the absence of succinic acid, apparently differs from the other organisms in part of its metabolic makeup.

Development of synthetic media "optimal" for growth. Although the basal medium (TABLE I) with a nitrogen source supported continued growth of most of the fungi, it was not qualitatively satisfactory for certain organisms (TABLE IV) or quantitatively optimal for any species. It was of interest to develop, for some of the organisms, synthetic media which were at least quantitatively optimal as regards essential nutrients (i.e., constituents of the original basal medium with glutamic acid as the source of nitrogen), using the general techniques given under Materials and Methods.

For determining the optimal concentration of each nutrient in the presence of others, the "triangle" method (e.g., **23**) was first used. With this method, concentrations of three nutrients are varied simultaneously, while keeping the concentrations of other constituents constant. This is repeated, using three other factors as variables, until the optimal concentrations of all have been determined with the desired degree of accuracy.

In the triangle method the variables are run only in groups of three,

and the interaction of each nutrient of the complete medium with all of the others cannot be studied at the same time. Using this method, however, it was found that varying the concentrations of the trace elements in the original basal medium had relatively little effect on growth, which left only six factors (glucose, glutamic acid, thiamine, potassium phosphate, magnesium sulfate and pH) to be intensively studied quantitatively. It was convenient and time-saving to study these six factors at the same time, with experiments set up like two superimposed triangle systems. Hence, the six-variable system was used for the rest of the studies to determine, simultaneously, the optimal concentration of each of these six variables in the presence of the others, keeping the amounts of trace elements constant. Using this procedure we developed, for five brown-rot fungi and four white-rot fungi, media which were quantitatively more nearly optimal than the original basal medium. The composition of these media, together with the amount of growth obtained in each compared with mycelial weights in the original basal medium, are shown in TABLE V.

TABLE V

SYNTHETIC MEDIA, QUANTITATIVELY OPTIMAL IN ESSENTIAL NUTRIENTS, FOR GROWTH OF CERTAIN BROWN ROTS AND WHITE ROTS

Constituent	A Original basal medium (TABLE I)	B Composition of media optimal for indicated brown rots				
		P. palustris	*L. trabea*	*D. quercina*	*T. serialis*	*F. subroseus* (20)
Glutamic acid*	0.012	0.1	0.02	0.04	0.12	0.08
Glucose*	1.00	12.0	8.0	10.0	16.0	10.0
KH_2PO_4*	0.15	0.5	0.4	0.4	0.5	0.6
$MgSO_4 \cdot 7H_2O$*	0.05	0.05	0.1	0.1	0.05	0.04
Thiamine**	1.00	2.0	4.0	3.5	1.5	2.5
Trace elements**— B, 0.10; Cu, 0.01; Fe, 0.05; Mn, 0.01; Mo, 0.01; Zn, 0.07		Same as original basal medium (A)				
Initial pH	5.0–5.5	5.2	4.4	4.8	4.8	4.2
		Growth in media				
Culture age in A and B, days		14	7	14	7	14
Growth in A, mg		105	78	52	69	74
Growth in B, mg		402	652	482	789	298
Growth increase (B/A × 100), percent		382	836	930	1145	403

* Expressed in percent. For glutamic acid, as *percent nitrogen*.
** Expressed as mg/l of medium.

TABLE V—*Continued*

Constituent	A Original basal medium (TABLE I)	B Composition of media optimal for indicated white rots			
		F. geotropus	*P. tulipiferus*	*A. mellea*	*L. tigrinus*
Glutamic acid*	0.012	0.12	0.14	0.06	0.10
Glucose*	1.00	4.0	20.0	14.0	12.0
KH_2PO_4*	0.15	0.8	0.4	0.3	0.6
$MgSO_4 \cdot 7H_2O$*	0.05	0.025	0.1	0.2	0.04
Thiamine**	1.00	1.5	1.0	3.0	2.0
Trace elements**— B, 0.10; Cu, 0.01; Fe, 0.05; Mn, 0.01; Mo, 0.01; Zn, 0.07		Same as original basal medium (A)			
Initial pH	5.0–5.5	5.2	6.0	5.0	5.4
		Growth in media			
Culture age in A and B, days		7	14	21	7
Growth in A, mg		125	96	25†	90
Growth in B, mg		1350	552	925	924
Growth increase (B/A × 100), percent		1080	575	3700	1025

* Expressed in percent. For glutamic acid, as *percent nitrogen*.
** Expressed as mg/l of medium.
† Grew after a period of adaptation, i.e., continued serial subculture.

It is apparent that in the media in TABLE V, which are "optimal" only in *essential* nutrients, there were large increases in the amounts of growth compared with the original basal medium—from 4-fold to 37-fold, depending upon the organism. The growth of *A. mellea* was especially striking, as this organism originally did not grow in the standard basal medium, and produced only small amounts of mycelium in malt extract (TABLE II; see also discussion of special growth conditions for *A. mellea* in preceding section). Also, it is noteworthy that some of the organisms produced a dry weight of mycelium as great as about 1 gm per 70 ml of medium. From the data obtained in developing these optimal media, it was found that in general the factors most critical in increasing growth were the concentrations of glucose, nitrogen and potassium phosphate, and to some extent the proper ratio of potassium to magnesium. The optimal concentrations of these factors varied with the species of organism.

For the four organisms in TABLE V for which 7-day growth data in the optimal synthetic media are available—*L. trabea, T. serialis, F. geotropus* and *L. tigrinus*—a comparison may be made with the respective 7-day mycelial weights in 1.0 percent malt extract solution (TABLE II)

and in the standard synthetic medium containing casein hydrolyzate (TABLE III). Such comparison shows that the amounts of growth in the optimal media were from 6 to 15 times as great as in the other media, although the latter have often been considered nutritionally "best" for growth.

Miscellaneous studies on amino acids. Data were completely lacking on the relation of isomerism to the utilization of amino acids by the wood rots. This question was not investigated thoroughly, but a few observations were made relative to certain of the compounds used in TABLE III. If only the L-isomer of an amino acid is utilized by an organism, the amounts of growth should be the same for the L- and the DL-forms when the latter is used in double the total nitrogen concentration of the former, assuming that the amount of growth is proportional to the L-nitrogen concentration. To determine the utilization of L- and DL-forms of amino acids, each of ten organisms was cultured, at optimum pH, in the standard basal medium with L- and DL-tryptophane and L- and DL-leucine at various levels above and below (0.0008–0.960 percent nitrogen) the concentrations routinely used previously (total nitrogen of 0.012 percent for L-isomers and 0.024 percent for racemates). The organisms used were *P. tulipiferus, P. palustris, L. trabea, F. annosus, T. serialis, P. monticola, L. tigrinus, P. anceps, P. rubescens,* and *P. gigantea.* Third-transfer dry mycelial weights were determined in the usual way. It was found that within the above range of nitrogen concentrations, only the natural (L-) isomer of tryptophane and of leucine appeared to be assimilated; the amount of growth of each organism was directly proportional to the L-, i.e., "utilizable," nitrogen concentration. These results suggest that isomerism plays a part in the utilization of amino acids by fungi. The data indicate also that the concentration of nitrogen in the basal medium (TABLE I) was not necessarily optimal, and may have been a limiting factor for the growth of certain organisms. (It was for the reason just shown that racemic mixtures (DL-) of amino acids were used (TABLE III) at a total nitrogen concentration (0.024 percent) twice that of L-amino acids and other nitrogen compounds (0.012 percent), i.e., on a comparable "utilizable" nitrogen basis.)

Utilization of amino acids as carbon sources was studied with *P. palustris* and *P. tulipiferus,* in the basal medium minus glucose, but with each single amino acid in TABLE III as the sole source of nitrogen and carbon. Growth in serial subculture did not take place with either organism in any amino acid. Similarly, utilization of L-glutamic acid as a nitrogen and carbon source was investigated with each of the

organisms in TABLE II, but none of the species so utilized this compound in continued subculture.

DISCUSSION

One or more of several factors may be responsible for the poor growth often observed in synthetic media—hydrogen ion concentration; buffer action; redox potential; osmotic concentration; lack of certain minerals, vitamins or other essential metabolites; inadequate kinds and amounts of carbon or of nitrogen, or an unfavorable quantitative balance of constituents. In our studies sensitivity to hydrogen ions was found to be responsible for the poor growth of *P. radiculosa;* for *P. vaillantii* and *F. officinalis* certain essential metabolites were lacking in the standard synthetic medium; growth of *A. mellea* was markedly influenced by both the redox potential and an unsuitable quantitative balance of the components of the standard basal medium. This last factor assumed outstanding importance in the development of synthetic media more nearly optimal for growth. For nine species it was possible, by determining the approximately optimal concentrations of the components of the standard basal medium, to develop media which supported several-fold more growth than 1.0 percent malt extract, casein hydrolyzate or the standard basal medium. The question of optimal concentrations of synthetic medium constituents has received little previous attention so far as the wood-rotting Basidiomycetes are concerned; most studies have dealt with specific components qualitatively.

Despite the commonly observed nutritional superiority of a complete series of amino acids, as in casein hydrolyzate, our results showed as much growth, on the average, with valine or with glutamine singly in the standard basal medium as with the hydrolyzate (TABLE III); several organisms produced significantly more growth in other single amino acids. While the reason for this is not clear, presumably certain environmental factors were more nearly optimal for assimilation of the single compounds than for the mixture of amino acids. Also, in this connection, it is of interest to note that none of the amino acids was "essential"; none was required as such, but each functioned merely as an available source of nitrogen.

It should be noted that our negative results as regards utilization of nitrate and nitrite nitrogen apply only under the "standard" conditions employed. No investigation was made of factors known to affect utilization of these compounds, such as pH (**5**, **20**), molybdenum (**30**), prolonged incubation (**8**), or the synergistic action of organic acids

(1, 8, 19). The latter phenomenon was demonstrated with our organisms, in that growth in the basal medium containing ammonium chloride took place in the presence but not in the absence of succinic acid. The same acid enhanced growth in other ammonium salts.

It is clear that, on the equivalent "utilizable" nitrogen basis used in these experiments, the various nitrogen sources were not assimilated to the same extent by the wood-rot fungi. Quantitative differences in assimilation have been a general finding in studies of fungal nutrition. Presumably both environmental factors and intrinsic differences in molecular structure may be involved in differences in utilization of nitrogen compounds. Steinberg (30), for example, found that if the concentrations of essential components of the basal medium were made optimal, ammonium nitrogen, nitrate nitrogen and organic nitrogen (urea, asparagine) were equivalent in value for the nutrition of *A. niger*. On the other hand, in studying the assimilability of amino acids by *A. niger*, Steinberg (31) found large difference in utilization on an equivalent total nitrogen basis. He considered these to be intrinsic differences, related not to isomerism but to chemical structure; for example, to the length of the carbon chain or to the position of the amino group. Other investigators also have shown that all amino acids are not of equal value in fungal nutrition (5, 16, 18, 20). Sulfur-containing amino acids (cysteine, cystine, methionine) usually have been found to be poor nitrogen sources (18, 29); cysteine was actually inhibitory for *A. niger* (31) under the conditions used. In our experiments the explanation of the apparent inhibitory action of cysteine lay in the low O-R potential which it produced in the synthetic medium. The effect of Eh on the utilization of nitrogen compounds by higher fungi apparently has been little investigated.

Generally, chemical structure has been considered to be the main intrinsic factor determining assimilation of amino acids (5, 31). Isomeric configuration has, until recently, received little attention, but investigations with a few organisms point to better or exclusive assimilation of the natural isomers (13, 14, 24, 36). Our results on the effect of isomerism strongly suggest the importance of this factor and, other conditions being the same, indicate that the degree of utilization of amino acids varies with their chemical structure.

SUMMARY

A quantitative comparative study was made of the growth and nutrition—particularly the utilization of known nitrogen compounds—of

common species of wood-destroying Basidiomycetes, both brown-rot and white-rot types, in submerged (shake) culture in synthetic media. Growth curves of 42 species in malt extract solution and in a synthetic medium showed that the maximum amount of growth of most forms was attained in about two weeks; usually growth was virtually complete in one week. Acid invariably was formed, with pH values as low as 1.5 being reached.

Two species of the 42 had no vitamin requirements; most of the others were deficient only for thiamine. All but four species grew in a standard synthetic glucose-mineral medium containing thiamine and any one of a variety of organic or inorganic sources of nitrogen. Of the four species which did not grow in the *standard* synthetic medium, two had vitamin requirements in addition to thiamine; one of the other two required a medium either of lower O-R potential than the standard or of a quantitatively different composition, and the other was especially sensitive to the concentration of hydrogen ions resulting from its growth. None of the organisms required *organic* nitrogen, although usually growth was greater in organic nitrogen than in ammonium salts, the known nitrogen compounds being used on a comparable "utilizable" nitrogen basis.

For 15 representative species of wood-rot fungi, statistically-significant differences in the order of average growth-supporting ability of single amino acids compared with casein hydrolyzate in the basal medium were as follows: casein hydrolyzate, DL-valine, glutamine > DL-aspartic acid, L-glutamic acid, L-arginine, L-asparagine, glycine, L-proline, DL-alpha alanine, DL-ornithine > L-hydroxyproline, L-tryptophane, DL-serine, DL-threonine, L-leucine, DL-phenylalanine, DL-methionine, DL-isoleucine > L-histidine, DL-norleucine, L-tyrosine, L-cystine > beta alanine, L-lysine, L-cysteine. The L-cysteine medium supported growth only when its Eh (initially + 350 mv) was raised to about + 450 mv by the addition of ascorbic acid; the Eh of all other media was + 400 to + 500 mv. With inorganic nitrogen compounds, urea or casein hydrolyzate singly in the basal medium, only the following statistically-significant difference in average growth-supporting ability was shown: casein hydrolyzate > ammonium nitrate, ammonium carbonate, ammonium sulfate, urea. Utilization of nitrite or nitrate nitrogen could not be demonstrated under the standard conditions used; growth in ammonium chloride medium took place in the presence but not in the absence of succinic acid. There were no significant differences between the brown-rot fungi as a group and the white-rot species, on the above nutritional bases.

Differences in utilization of amino acids seemed to be related to molecular structure other than isomerism, when the latter was taken into account experimentally. The simple monoamino, monocarboxy amino acids of short chain length averaged significantly greater amounts of growth than did those of longer chain length; the short-chain acids containing an OH group were quantitatively nutritionally similar to the simple ones of longer chain length. Sulfur-containing amino acids were, on the average, poorly assimilated. Monoamino, dicarboxy acids (except lysine, a poor nitrogen source) were utilized to about the same extent as the simple, short-chain forms. The aromatic amino acids were nutritionally similar to the aliphatic, sulfur-containing compounds; the heterocyclic forms varied from good to poor. Utilization of amino acids as carbon sources could not be demonstrated.

Synthetic media more nearly optimal for growth than the standard basal medium were developed for nine species, by making quantitative changes in the constituents of the standard medium. The new media supported several-fold more growth than did the standard synthetic medium, the casein hydrolyzate medium or 1.0 percent malt extract solution.

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THE EFFECT OF CORTISONE, SOMATOTROPHIC HORMONE, AND PIROMEN ON EXPERIMENTAL MONILIASIS IN MICE[1]

GEORGE H. SCHERR[2]

Piromen (Travenol Laboratories, Inc.) is a polysaccharide extract prepared from cultures of *Pseudomonas sp.* Its pyrogenic and other pharmacological properties have been described by Nesset *et al.* (**2**), Windle (**12**), Wittich (**14**), and others. Piromen possesses many properties of cortisone and ACTH and has been used successfully in various allergic disturbances (Wittich, **13**), yet certain of its activities differ markedly from those of cortisone (Stuart, **11**). Our studies (Scherr, **5**, **6**) have shown that cortisone could have either a deleterious or an efficacious effect on mice infected with moniliasis, depending upon certain conditions. Somatotrophic hormone (STH) used in conjunction with cortisone was partially successful in suppressing certain of the deleterious properties of cortisone without antagonizing certain of its efficacious effects (Scherr, **7**, **8**). Thus, female mice infected with moniliasis and treated with a mixture of cortisone and STH showed a less severe disseminated infection than a similar group infected but untreated or animals infected and treated solely with cortisone. Since the effect of cortisone and STH on experimental moniliasis is probably an indirect one and since many of the pharmacological activities of Piromen may be attributed to its influence on the adrenal cortex, it was felt that an investigation of the effect of Piromen, cortisone, and STH on moniliasis in mice might be pertinent.

MATERIALS AND METHODS

The materials and methods used here were essentially those previously described (**6**, **7**, **9**). The mice were Webster strain white mice approximately 18–20 grams in weight. Twenty mice comprised an experimental group.

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Treatment was instituted 24 hours after infection with *Candida albicans* strain ATCC *No. 11651.* Cortisone (Schering) was inoculated intramuscularly, 0.3 mg (0.05 ml) every 48 hr for the first two weeks and a doubled dose in the same volume thereafter; 0.15 mg (0.05 ml) STH (Frank W. Horner, Ltd.) was inoculated intramuscularly, daily for the first two weeks and a doubled dose in the same volume thereafter; Piromen was inoculated intraperitoneally at a dosage of 0.1 gamma (0.05 ml) per mouse every 48 hours for the duration of the treatment period. The vehicle for all these agents was the aqueous vehicle in which the cortisone is suspended. Mice treated solely with the aqueous vehicle received 0.1 ml intramuscularly every 48 hours. Uninfected animals were treated concomitantly with and similarly to the infected ones.

The method of computing the severity of infection and the mortality rate by "dissemination values" has been previously described by Scherr (**6, 7, 9**).

RESULTS

The data for this experiment are summarized in TABLE I. In accord with our previous method of evaluating severity of infection and mortality data by means of the mean dissemination value, all values of 6 or greater indicate that the animal died before the termination of the experiment.

Piromen markedly reduced the severity of the infection for male and female mice; a mean dissemination value of 7.6 for males was reduced to 1.4 and a value of 11.1 for females was reduced to 1.7 after treatment with Piromen. Cortisone reversed to a considerable extent this efficacious effect of Piromen for infected males but not for females. This difference in susceptibility of the two sexes may be ascribed to the fact that male mice are more susceptible to treatment with cortisone than female animals. This contention is supported by the fact that normal male mice are more susceptible to the toxic properties of cortisone (Scherr, **4**) and that an amount of STH which reduced the deleterious effect of cortisone for infected female mice was not effective for males (Scherr, **7**).

The mixture of Piromen and STH resulted in a higher mean dissemination value (4.4) for males than Piromen alone. As can be seen from TABLE I, STH enhanced the efficacious effect of Piromen on infected female mice, since the mean dissemination values for infected female mice untreated, treated with Piromen, and treated with Piromen and STH were 11.1, 1.7, and 0.5, respectively. However, cortisone was

able to antagonize this effect of STH so that infected female mice treated with Piromen, STH, and cortisone showed a mean dissemination value of 4.2.

The dosage of cortisone used here is not toxic to normal mice and our previous findings (**5, 7, 8**) have shown that it should have little effect on these infected male and female mice.

TABLE I

THE EFFECT OF CORTISONE, SOMATOTROPHIC HORMONE (STH), AND PIROMEN ON SYSTEMIC MONILIASIS IN MICE

(Duration of experiment—28 days)

Treated with:	Untreated		Piromen and STH		Piromen		Cortisone and Piromen		Cortisone, STH, and Piromen	
Sex	M	F	M	F	M	F	M	F	M	F
	DISSEMINATION VALUES									
Dissemination Values	0	0	0	0	—	0	1	0	0	0
	2	0	0	0	0	0	2	0	0	0
	2	1	0	0	0	0	2	0	1	0
	2	2	0	0	0	0	2	0	1	0
	2	2	0	0	0	0	2	1	2	0
	2	2	2	0	0	0	2	1	3	0
	2	2	2	0	0	0	2	1	3	0
	2	2	2	0	0	0	3	2	3	1
	2	3	2	0	0	0	3	2	3	1
	3	3	2	0	0	0	3	2	3	2
	3	3	2	0	0	0	3	2	3	2
	3	4	2	0	0	0	3	2	3	3
	4	17	2	0	1	0	3	2	4	3
	4	20	3	0	1	0	4	3	5	4
	9	23	3	0	2	1	4	3	5	4
	21	24	3	1	2	2	4	3	5	4
	21	24	9	1	2	2	5	3	7	4
	22	27	11	2	2	2	5	3	8	11
	22	31	15	2	6	7	15	3	16	22
	24	31	27	3	11	19	26	4	23	23
Mean	7.6	11.1	4.4	0.5	1.4	1.7	4.7	1.9	4.9	4.2

Although not shown in these data, it has been determined on other occasions that the aqueous vehicle has no significant effect on the course of this infection, either for male or female mice.

The relatively large number of male and female animals treated with Piromen which were free of any visible pathology at the end of the treatment period is more significant as a reflection of the beneficial effect of this treatment than the decrease in mortality of these animals. The

differences in susceptibility to these treatment schedules which we can ascribe to the sex of the animals are clearly indicated by the data for the infected male and female mice treated with Piromen and STH and those treated with Piromen and cortisone (TABLE I). In accord with these sex differences it has been shown by Scherr (**10**) that estradiol and testosterone injected into normal and infected male and female mice play a major role in altering the response of these animals to treatment with cortisone and/or STH.

The effect of these same agents and combination of agents on uninfected male and female mice was essentially uninteresting and with no significant toxicity excepting mortalities of 25%, 50%, and 20% for male mice treated with Piromen, male mice treated with STH, and female mice treated with cortisone and STH, respectively.

DISCUSSION

The beneficial effect of Piromen on infected male and female mice cannot be attributed to the pyrogenic activity of this preparation *per se* because it has been repeatedly demonstrated that an increased environmental temperature will increase the pathology and mortality of mice infected with moniliasis (**6**, **9**).

The therapeutic effect of Piromen might be due to properties simulating those of cortisone without the toxicity of cortisone. The fact that cortisone may have an efficacious effect on different infectious diseases and/or intoxications under certain conditions is supported by the experimental data of Duffy and Morgan (**1**), Robinson *et al.* (**3**), and Scherr (**6**). The data presented here support the relationship between the activity of Piromen and its effect on the adrenal cortex by demonstrating the influence of cortisone and STH on the effectiveness of Piromen in suppressing moniliasis in mice. The toxic effect of Piromen for normal male mice but not for female mice might also represent the influence of Piromen on the adrenal cortex, in line with our contention that male mice are more susceptible to the toxic effects of cortisone than female mice.

The low toxicity of Piromen reported clinically, and the efficacious effect reported here, would support the worth of a clinical evaluation of this regimen of therapy for systemic moniliasis. In addition, since the effect of Piromen on the *Candida* organisms is probably an indirect one through its effect on the host, it might be pertinent to explore the effectiveness of Piromen for similar infectious organisms.

SUMMARY

Piromen injected intraperitoneally into male and female mice infected with moniliasis in a dosage of 0.1 gamma per mouse every 48 hours served to suppress markedly the severity of the infection and mortality. Cortisone reversed this efficacious effect for male mice but not for females. The combination of Piromen and STH reduced the severity of the infection still further in female mice, but this apparent synergistic effect was antagonized when cortisone was introduced into the mixture. The implications of these data, especially for their clinical application, are discussed.

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A SEXUAL HORMONE IN GLOMERELLA [1]

C. H. Driver and H. E. Wheeler

Extensive cytological and genetical studies of *Glomerella cingulata* (Stonem.) Sp. & v. Schr. have revealed a number of unusual phenomena related to the sexual process in this fungus. One of these is the behavior of certain cultures in matings. When self-fertile cultures are mated with cultures which are self-sterile, or nearly so, perithecia obtained from the line of contact between the paired cultures are of three types, 1) those that give rise to cultures of the self-fertile parent only, 2) those that give rise to cultures of both the self-fertile and self-sterile parental types, and 3) those that give rise to cultures of the self-sterile type only (**11**). Perithecia of the first type can be attributed to selfing of the self-fertile parent and those of the second type have been demonstrated to be the result of cross-fertilization between the mated cultures (**3**, **11**). However, the origin of perithecia of the last type has been a perplexing problem, especially in view of the fact that in some cases these represent a majority of the perithecia formed.

Certain aspects of this problem of "induced selfing" of cultures which are either nearly or completely self-sterile have been considered in previous papers (**3**, **6**, **11**). Since the phenomenon occurs only in matings in which one member is a highly self-fertile culture, it has been suggested that a hormone-like substance might be involved. This hypothetical substance, produced by the self-fertile member of the mated pair, would be capable of rendering self-sterile cultures temporarily self-fertile. This investigation reports the results of attempts to demonstrate the existence of such a substance.

EXPERIMENTAL PROCEDURE AND RESULTS

The cultures used were the following: (1) self-fertile, clumped-perithecial cultures (genotype A^+B^+), (2) nearly self-sterile, scattered-perithecial cultures (genotype A^+B^2) and (3) completely self-sterile, conidial cultures (genotypes A^1B^+ and A^1B^1). These cultures, which

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originated from an isolate of *G. cingulata* from *Ipomoea,* have been described in detail by Wheeler and McGahen (**11**).

Most of the experiments were designed to demonstrate a diffusible principle produced by clumped-perithecial cultures which would increase the fertility of nearly self-sterile, scattered-perithecial cultures. These two types of cultures were selected because in matings the latter consistently exhibited the phenomenon of induced selfing (**11**). The almost complete self-sterility of the scattered-perithecial culture type was first reported by Edgerton (**5**), who called this type "minus," and his observations have been confirmed by several other workers (**1**, **2**, **8**). Campa (**2**) recently made a quantitative study of the fertility of cultures of this type grown on a variety of natural and synthetic media. She selected the best developed perithecia from cultures grown on the most favorable medium (oat meal agar) and found that only 2.2 percent were fertile. Campa also reported that 76 percent of the perithecia produced by mating clumped- and scattered-perithecial cultures on this same medium were fertile and that, of these, 23 percent gave rise to the latter culture type only. This illustrates the magnitude of the effect, termed induced selfing, which occurs in matings of these two types of cultures.

Three different approaches were used in attempts to demonstrate an active principle produced by clumped-perithecial cultures which would induce selfing in scattered-perithecial cultures.

One of these involved growing the two cultures on opposite sides of a semi-permeable membrane, a method which has been used successfully by Raper (**7**) to demonstrate sexual hormones in Achlya. In a second approach one of the cultures was labeled with C^{14} as described by Wheeler (**9**) in the hope that this would allow determination of the origin of individual perithecia formed by mating the two cultures. Although a large number of experiments were performed using these two methods, no conclusive evidence of a substance capable of inducing selfing in scattered-perithecial cultures was obtained. The fungus readily penetrated most of the membranes tested, and, in cases in which membranes were not penetrated, only slight changes in the fertility of scattered-perithecial cultures were observed. Data obtained from matings between clumped- and scattered-perithecial cultures in which one member of the mated pair was labeled with C^{14} indicated that in general most of the cross-fertilized perithecia were produced by the clumped culture and that perithecia yielding only one culture type originated from the parent of that type. However, the method was not sufficiently sensitive to allow unequivocal determination of the origin of individual isolated perithecia.

In a third approach, scattered-perithecial cultures were treated with sterile filtrates of liquid media on which clumped-perithecial cultures had been grown. After preliminary tests had shown that such filtrates contained a substance which induced selfing in scattered-perithecial cultures, a series of experiments were conducted to determine the conditions most favorable for the production of this substance. The most satisfactory procedure developed was as follows: Clumped-perithecial cultures were grown at room temperature for at least nine days on a liquid oatmeal

TABLE I

THE EFFECT OF FILTRATES FROM CLUMPED-PERITHECIAL CULTURES ON SELF-FERTILITY IN SCATTERED-PERITHECIAL CULTURES

Test No.	Number of perithecia			
	Examined	Fertile	Per cent fertile	Tested for type
D40A	51	48	94	41
D50B	35	20	57	10
D50D	20	10	50	10
D56B	25	15	60	15
D56C	110	90	82	45
D57A	54	34	63	24
D58A	31	19	61	11
D59A	20	12	60	11
D59B	30	19	63	10
D60A	35	15	43	10
D60C	45	31	69	10
D62A	30	21	70	10
D64AB	20	13	65	10
D65	20	17	85	10
TOTALS	526	364	69	227
Control[1]	600	15	3	15
Control[2]	488	11	2	11

[1] Untreated scattered-perithecial cultures.
[2] Scattered-perithecial cultures treated with 1 ml of oatmeal decoction.

decoction.[2] The growth medium was then filtered through an ultra-fine (bacteriological grade) fritted glass filter and 0.5 to 1.0 ml of the sterile filtrate was pipetted onto 1–4-day-old oatmeal agar plate cultures of the scattered-perithecial type. After 15 days incubation at 25–27° C, the treated cultures were analyzed for the presence of fertile perithecia. This was done by locating the best developed perithecia with a dissecting microscope, picking them up with a fine steel needle, crushing each one

[2] Oatmeal decoction was prepared by soaking 60 g of rolled oats in 500 ml of tap water at 60–70° C for 20 minutes, straining through cheesecloth and bringing the total volume to 1 liter. Oatmeal agar was prepared in the same way except that 17.5 g of agar was added.

in a drop of sterile water in a petri dish and examining the dish under a compound microscope to determine the presence of ascospores. Ascospores from approximately two-thirds of the fertile perithecia were cultured to make certain that they resulted from selfing of the treated culture. Controls for these experiments included scattered-perithecial cultures untreated and treated with liquid oatmeal decoction upon which no culture had previously grown. All filtrates were checked for sterility before they were used.

The results of 14 different experiments in which filtrates from clamped-perithecial cultures were applied to scattered-perithecial cultures are presented in TABLE I. These data show that the filtrates had a marked effect upon the numbers of fertile perithecia produced by the treated cultures. The highest degree of fertility obtained by these treatments was 94 percent in test D40A, the lowest was 43 percent in test D60A, and the average in all tests was 69 percent. Compared to the controls, in which values of 2 and 3 percent were obtained, the treated cultures exhibited a striking increase in self-fertility.

Ascospores were cultured from 227 of the fertile perithecia obtained in the tests in TABLE I. In all but four cases, these gave rise to typical, nearly sterile, scattered-perithecial cultures only. A number of these cultures were transferred several times and observed over a period of six months. During this time they remained true to type. This indicated that the temporary change in fertility induced by the use of filtrates could not be attributed to genetic changes occurring in the treated cultures. Ascospores from the four exceptional perithecia gave rise to both clumped- and scattered-perithecial types. These probably resulted from reverse mutations which have been shown to occur occasionally in scattered-perithecial cultures (**4**).

PRODUCTION AND NATURE OF THE ACTIVE PRINCIPLE

In general, the conditions most favorable for the production of fertile perithecia were also found to be most favorable for the production and activity of the substance responsible for induced selfing. Media, having oat flakes as the basic ingredient, have been shown to be much more favorable for the production of fertile perithecia in *Glomerella* cultures than any other tested (**2, 5**). The only liquid medium upon which clumped-perithecial cultures fruited abundantly was oatmeal decoction and filtrates from such cultures were the only ones which were consistently highly active in inducing selfing. Tests were made with a number of liquid media upon which clumped-perithecial cultures failed to fruit. Filtrates from these were completely inactive. Similar negative results

were obtained with filtrates from nonperithecial (conidial) cultures and from nearly self-sterile, scattered-perithecial cultures grown on oatmeal decoction.

Another series of tests revealed that filtrates from clumped-perithecial cultures grown for less than 9 days or for more than 24 days on oatmeal decoction were completely inactive and that cultures 10–15 days old yielded the most highly active filtrates. Examination of these cultures showed that perithecia containing mature ascospores were not present until after 8 days of growth and that these continued to form until the cultures were about 20 days old. These findings indicated that the substance responsible for induced selfing is produced only while fertile perithecia are developing.

Scattered-perithecial cultures which varied in age from 1 to 15 days were treated with a single lot of filtrate which was known to be highly active. The greatest amount of induced selfing occurred when 1–4-day-old cultures were treated and very little or no selfing could be induced by treating cultures which were more than five days old. These results seemed to indicate that the substance responsible for induced selfing affected a specific stage in the sexual process and that it was not effective after that stage had passed.

Recapitulation of this phase of the study reveals that induced selfing occurred only when scattered-perithecial cultures were treated at the appropriate time with filtrates obtained from clumped cultures which were actively producing fertile perithecia. When these conditions were not satisfied, only negative results were obtained. These data strongly indicate that induced selfing is brought about by the action of a specific hormone-like substance rather than as a result of some nonspecific or nutritional effect of the filtrate on the treated culture.

DISCUSSION AND SUMMARY

Although previous studies of *Glomerella, Neurospora,* and *Bombardia* have indicated that diffusible substances are active during the sexual process in these organisms, Raper (**7**) has pointed out that in the Ascomycetes sexual hormones similar to those found in *Achlya* have not been conclusively demonstrated. Raper (**7**) defines sexual hormones as "specific substances secreted by the plant itself and which are essential for the initiation and coordination of the sexual process, or stages thereof, in the individual or between individuals of the same species." On the basis of this definition, the active principle responsible for induced selfing in *Glomerella* cultures may be properly termed a sexual hormone.

The self-fertile, clumped-perithecial (wild-type) cultures and the nearly self-sterile, scattered-perithecial (mutant) cultures used in this study differ by the single gene pair, B^+; B^2 (**11**). Cultures of the latter type, which carry B^2, produce an abundance of perithecia that reach mature size, and the self-sterility of such cultures results from nuclear disintegrations which occur in most of the asci just prior to or during karyogamy (**10**). It has been suggested that mutant genes such as B^2, which affect specific steps in the sexual process, act as partial or complete genetic blocks and that the sterility of cultures that carry such genes results from their inability to synthesize all of the substances required for the completion of the sexual process (**10**). On the basis of this interpretation, the active principle or sexual hormone demonstrated in this study represents a specific substance required for karyogamy. The fact that the active principle responsible for induced selfing is produced only by cultures actively undergoing karyogamy and the fact that it is effective only if applied to cultures before they reach this stage of development provide support for this interpretation.

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COMPATIBILITY AND VARIABILITY IN MERULIUS AMERICANUS

SHUH-WEI HWANG[1]

(WITH 4 FIGURES)

Variability within a species is a fact that has been demonstrated for many fungi (**3**, **4**, **5**) although there are but few records of variations among both monokaryon and dikaryon strains of the more important wood-destroying fungi. In 1924, Henry Schmitz (**8**) reported on *Fomes pinicola* Fr. from the physiological specialization aspect. Kaufert (**6**) reported that monokaryotic mycelia of *Pleurotus corticatus* Fr. grew only about half as fast as "dikaryotic mycelia." Verrall (**9**) found that dikaryotic lines of *Fomes igniarius* (L.) Gill. grew faster than their monokaryotic components. The species was found to be variable in a number of other cultural, physiological, and morphological characteristics. Kaufert (**6**) and Verrall (**9**) found for *F. igniarius* that the differences in growth rate of dikaryotic and monokaryotic lines applied to rate of decay of infected wood as well as to mycelial growth on agar.

The studies herein reported are concerned with variability within the species *Merulius americanus* Burt.[2] Monokaryotic and dikaryotic phases were compared as to cultural appearance and rate of growth. Similar studies also were made among isolates from these monokaryotic and dikaryotic strains. Sexual compatibilities of the haploid lines were determined between and within each of the three specimens.

MATERIALS AND METHODS[3]

Specimen *97419* (collected October 25, 1950) and specimen *97439* (collected January 15, 1951) were collected by Mr. R. W. Davidson of

[1] Mycologist, U. S. State Department grantee; research performed under the supervision of Dr. Carl Hartley, Principal Pathologist, and Mr. Ross W. Davidson, Pathologist, Division of Forest Pathology, U.S.D.A., Plant Industry Station, Beltsville, Maryland.

[2] This is the fungus reported under this name by Davidson and Lombard (**2**). It is believed to be the same as *M. himantioides* as referred to by other authors.

[3] The materials and facilities were furnished by the Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

the Division of Forest Pathology from pine logs at Beltsville, Maryland. The third specimen, *97446*, was collected in Maine by Dr. J. D. Diller, of the Division of Forest Pathology, in February, 1951. These are specimen numbers in the Forest Pathology Herbarium and will be identified as I, II and III, respectively.

Cultures made from these specimens, both monokaryons and dikaryons, were kept on 2% Fleischmann's diamalt agar medium at room temperatures during an entire year of study. No artificial means were used to induce variations.

Monosporous isolates were obtained directly from fresh spore prints from the above three specimens. Spores from sporophores were deposited on dry sterile filter paper. Small quantities of spores from the filter paper were washed into sterile water. The spore suspension was then poured over agar in Petri dishes. After four or five days germinating spores were picked out singly with the aid of a dissecting microscope, after first being examined under a compound microscope. Fifty-two monosporous isolates were obtained from specimen I, twenty-eight from II, and three from III. Each of them was numbered by an Arabic numeral. The Roman numeral preceding the Arabic numeral, as I–4, II–5, III–101, etc., indicates from which of the three specimens each haplont was isolated.

COMPATIBILITY STUDY

Some single-spore isolates from the three specimens were picked at random for the following study. Single-spore cultures from one specimen were paired in all possible combinations among themselves. When clamp-connections were seen in any of the combined colonies, it was recognized that the pairs were compatible components and that dikaryotization had taken place.

The results of mating experiments are summarized in FIG. 1. This reveals that monosporous cultures fall into four groups (AB, ab; Ab, aB), dividing into two units. The members within one unit are compatible pairs. Inter-unit crossing produced no clamp-connections. Further study showed that the three specimens studied are interfertile. The results of all these crosses produced only two kinds of dikaryotic mycelia, (AB + ab) and (Ab + aB), out of the four kinds of monosporous cultures. For two haplonts to be compatible, they must differ in both A and B factors. Thus the dikaryotized hyphae will have a dikaryon AaBb. These findings agree with those for *Coprinus lagopus* Fr. by Buller (1). Therefore, the three specimens of *M. americanus* are of the same species with heterothallic and tetrapolar genetic characters.

A deviation in matings, however, was observed. When an oidium-dominated haplont grew side by side with its compatible normal mycelial haplont, clamp-connections would not develop in the oidial-dominated colony, while clamp-connections would develop as usual in the mycelial colony. This may be illustrated by the mating between I–26 and II–21. II–21 is a normal non-oidial haplont, while its compatible component I–26 is an oidial haplont. After these two grew together on malt agar,

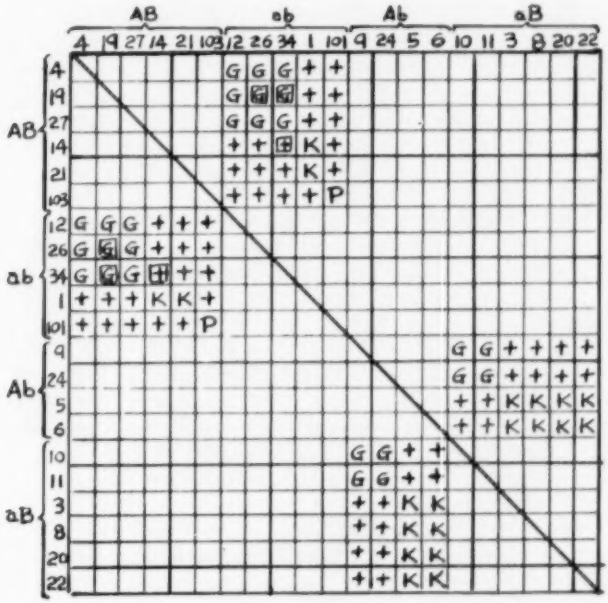

FIG. 1. Results obtained by pairing monosporous mycelia from each specimen and between the three specimens. G, K, and P, positive pairings from specimens I, II and III, respectively. + positive pairings between specimens; □ theoretical positive pairings, but no clamp-connection mycelia developed, because of oidial condition.

clamp-connections could be found either at the line of merging hyphae or on the mycelium at the side of II–21 near the merging line. A few days later, clamp-connections could also be found in any part of colony II–21. But no clamp-connections developed on colony I–26, neither near the merging line nor on the periphery of the mat where oidia were not so abundant.

Similar results were also obtained from pairing of II–5 × II–8, II–1

× I–19, II–1 × I–27, II–8 × I–24. Haplonts II–5, I–19, I–27 and I–24 are oidium-dominating cultures. No clamp-connections developed in the colonies of these isolates when matched side by side with their compatible normal mycelial haplonts II–8 and II–1. Clamp-connections developed on the latter two mycelial haplonts as expected.

Another crossing was made between an oidial and a non-oidial culture. Inoculum of haplonts II–8 and I–24 was planted on the same dish, 1 and ½ cm apart. When these colonies met and clamp-connections developed on the hyphae at the merging line, a small piece of agar was cut from it and transferred to a fresh plate. Care was taken to make sure that this inoculum contained mycelia from both colonies. A transfer was also made from each colony to two different agar plates. The inoculum was cut at an equal distance from the merging line from each colony.

The inoculum composed of both colonies developed into a normal dikaryotic mycelial culture, except that the small center was distinctly powdery. Under microscopic examination, clamp-connections were found in the mycelium from the region where oidia were absent. Oidia were found and clamp-connections were absent in the powdery center.

The plate inoculated with mycelial colony II–8 developed into a dikaryotic mycelial culture with clamp-connections and no oidia. The plate inoculated with oidial colony I–24 retained its powdery appearance with no clamp-connections. Similar results were obtained from a cross between the oidial haplont II–5 and the normal mycelial haplont II–8 (FIG. 2, *A*).

A similar observation also was made on a crossing between I–19 and III–101. III–101 is a non-oidial haplont and I–19 is an oidium-dominated haplont. Clamp-connections were formed at the line of the merging hyphae or on the hyphae at the side of colony III–101; but they were not found on I–19's side (FIG. 2, *B*).

The following crosses were made between two oidial haploid cultures. Theoretically the pairings between I–19 and I–26 and II–14 and I–34 should result in dikaryotization and are shown in FIG. 1 as marked squares. However, after they were paired or grew together, no clamp-connections were found in any part of these colonies or in the hyphae of the merging lines. They retained the same cultural characteristics as their haploid parents. Therefore, their appearance is powdery, with thin narrow mycelial periphery. The oidia so completely dominate the entire colony that not even the mycelium in the periphery is free from their presence.

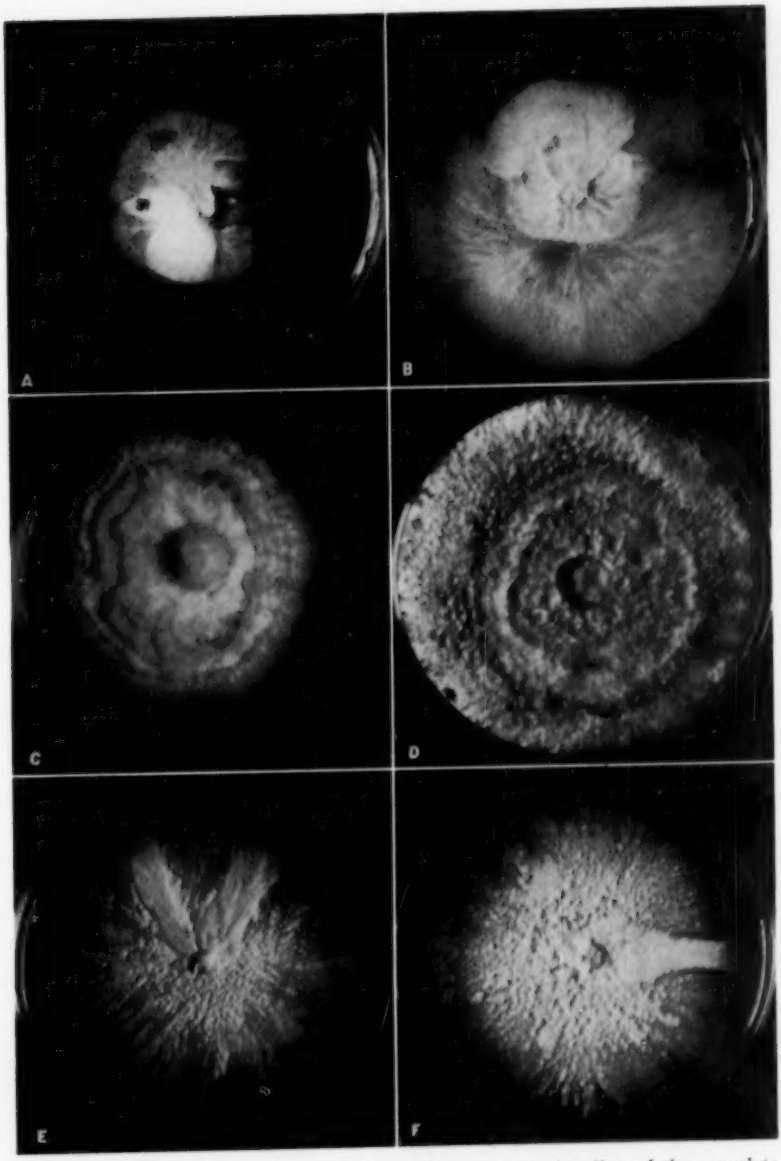

FIG. 2. a) 16-day-old first subculture from the merging line of the complete pairing between II-5 × II-8; b) between I-19 × III-101. Color zonations in a dikaryon culture 12 × 19; c) 15-day-old; d) 35-day-old. Oidial variant cultures, e) a 35-day-old dikaryon 4 × 34; f) a 42-day-old monokaryon II-2.

Further confirmation was made by crossings between I–34 and I–19, and I–34 and II–14. After the two components met, transfers were made from the merging line and from each of the two colonies to fresh agar plates, respectively. All of them developed into powdery cultures. Under microscopic examinations short-rod oidia were abundant; no clamp-connections were found in any of these subcultures. This evidence further supports the belief that the presence of oidia impedes the dikaryotization process.

Buller (1) used *Coprinus lagopus* to demonstrate how the compatible nuclei travelled and dikaryotized a haploid colony. A small piece of haploid mycelial mat was placed on a growing compatible colony; the nuclei from this mat invaded the mycelium of the compatible component and continued to move as the colony was continuing its growth. But if the colony reached the edge of the dish and ceased its growth before the compatible foreign nuclei arrived, that part of the colony would not be dikaryotized and, therefore, the mycelium in that region would bear no clamp-connections. In the case of oidial cultures of *M. americanus*, normal length hyphae are reduced to such an extent that it is difficult to find one not broken up into oidia. These short rod-shaped oidia may lie end to end. But, of course, the cytoplasm of each oidium does not form a continuous cyloptasmic stream as in the cells in the mycelium. According to Buller's (1) evidence, foreign nuclei need a continuous path of travel. Therefore, it is reasonable to presume that the haploid oidial colony could not be dikaryotized by its compatible component because of this physical barricade.

CULTURAL CHARACTERISTICS AND THEIR VARIATIONS

A normal dikaryotic culture of *Merulius americanus* is composed of a white, fluffy aerial mycelial mat. Clamp-connection hyphae are easily found by microscopic examination. No accessory spores are produced. Crystals are rarely seen in a fresh culture, but particles and crystals may be seen in old cultures.

In physical appearance a monokaryon culture cannot be distinguished from a dikaryon culture. Under microscopic examination it agrees with what has been described above for the dikaryophase, except that no clamp-connections will ever be found in a monokaryon culture. Therefore, the presence or absence of clamp-connections is a basic morphological feature by which it is possible to distinguish the two phases in a complete life cycle.

COLOR VARIATIONS

A rusty color (according to Ridgway (**7**), the color varies between "Onion-Skin Pink," "Terra Cotta," and "Testaceous") with a more or less definite pattern has been found among the normal white cultures. Color starts as yellowish or light pink in the young culture. The rusty coloring and the yellow zonations gradually develop to their full intensity as this young culture continues its growth, and they do not fade with age.

The dikaryotic culture I–4 × I–12 was one of the few that developed such a coloration pattern. The rusty color stained the large center of the mycelial mat, and faint pinkish patches spotted the wide white mycelial periphery band. These described characters reappeared in four successive transfers. In most of the cases, deeper color occurred in a culture when the inoculum came from the rusty-colored mat, although subcultures made from the spotted periphery would still develop into the same color pattern. Later crosses made between haplonts I–4 and I–12 have revealed similar characteristics. The predominating rusty center was sometimes alternated with one or two yellowish, incomplete rings while the white periphery remained spotty. The rusty ring pattern was also observed on the small free agar surface when subcultures were made from this cross on a test-tube agar slant.

Light pink or yellow spots were observed in the haplont I–12, but the color did not develop any deeper or into a distinct pattern. During this entire study the haplont I–4 remained as white as the rest of the normal cultures.

Such colors and patterns were also observed among the following dikaryons: I–12 × I–19 (FIG. 2, *C*, *D*), I–12 × I–27, I–9 × I–10, I–9 × I–11, I–10 × I–24; II–3 × II–6, II–3 × II–5; and III–101 × III–103. The deepest color occurred when monokaryon I–12 was one of the components of the dikaryon. Of the five dikaryons obtained from those new crossings made at different times between haplonts II–3 and II–5, all developed the same color patterns. Such characteristics reappeared in the subcultures of these newly formed dikaryons. Among the monosporous isolates, haplont II–3 was the only one besides I–12 to have scattered pale yellow patches develop on its mycelial mat.

The appearance of these colored variants among the normal white cultures was not very common. Yet because their occurrence was constantly linked to the dikaryophase from certain crosses it indicated that this is one of the genetic characteristics of *M. americanus*. The variation in the pattern and the intensity of the color further suggests that this characteristic may be governed by a set of genes. The reactions

of these genes showed sensitivity to the presence of other genetical and physiological factors. Therefore, their occurrence in mass-transferred cultures was rare. If they happened to show up, they could easily be mistaken for a temporary characteristic produced by high temperatures or some unknown environmental factor.

GROWTH RATE STUDY

FIG. 3 shows the average growth rates of 16 dikaryons and 50 monokaryons in 4, 7, 11, 14 and 21 days. The dikaryons tended to grow at

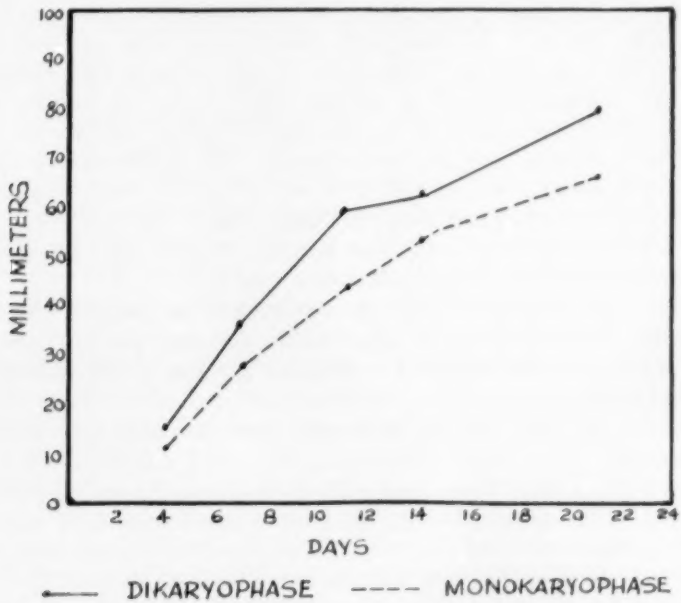

FIG. 3. Average growth rates of 50 monokaryons and 16 dikaryons at 4, 7, 11, 14 and 21 days.

a faster rate than the monokaryons. This distinction began in young, 4-day-old cultures and yet might continue at lessened rate as the cultures became older. For example, in seven days the average dikaryotic colony of II–22 × II–6 had reached three times the diameter of colonies of each parent, but in fourteen days it was less than twice such diameters. Also haplont II–5 is a slow grower and its compatible component II–3 is a normal grower, but their dikaryon II–3 × II–5 grew faster

than haplont II–3. A similar difference was found between the monokaryons I–19 and I–12 and their dikaryon I–19 × II–12.

The 16 dikaryons are divided into three groups. The members of the *a* group are slow growers, which came from slow-growing haploid parents, as *a*–1 and *a*–2, indicated in FIG. 4. In the *b* group, one of the monokaryotic parents is slow and one fast; as a result, two dikaryons (*b*–1 and *b*–2) are slow-growing and the other four (*b*–3 to *b*–6) are fast growers. For the *c* group, both monokaryotic parents are fast

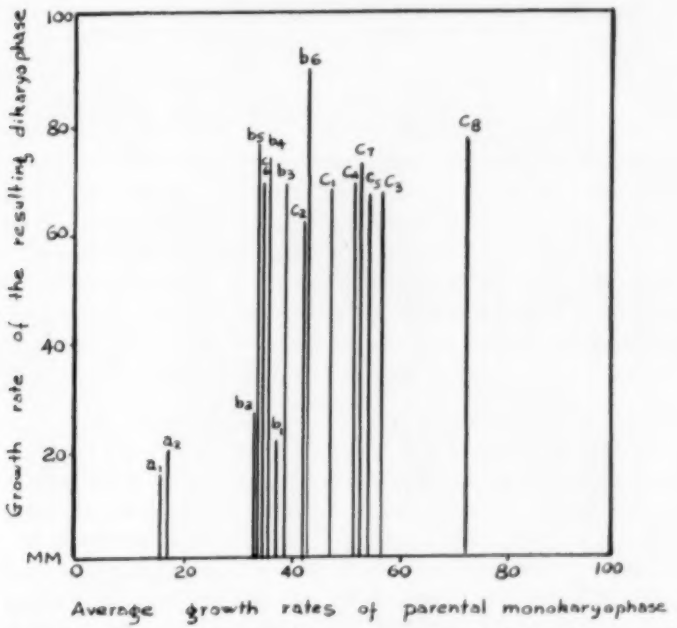

FIG. 4. Growth rates of 14-day-old monokaryons and their dikaryons, after TABLE I.

growers and all their dikaryotic progenies (*c*–1 to *c*–8) are fast. When both parents are either slow or fast growers, it is no surprise to find their offspring also respectively slow or fast. But it is interesting to find a ratio of 1:2 for slow against fast in the dikaryons of the *b* group, for each of which one of the parents is a slow grower. Since no back crossing has been made, the genetical constitution of this ratio is left unsolved.

In TABLE I, the measurements are shown for 7 and 14 days, re-

spectively. Ten millimeters of the colony diameter represented by the inoculum disk were deducted from the gross diameter so that the measurements shown are for the true growth of the organism during the test period. Most measurements represented the average growth rate of two culture plates; in a few cases only one measurement was taken

TABLE I

GROWTH RATES OF MONOKARYOTIC AND DIKARYOTIC CULTURES OF M. AMERICANUS
(Diameter of colony in millimeters)

Monokaryon isolate numbers	7 days after inoculation				14 days after inoculation				Ref. to Fig. 4
	Monokaryon Culture	Average of 2 monokaryons (b)	Resulting dikaryons (a)	Difference (a−b)	Monokaryon	Average of 2 monokaryons (b)	Resulting dikaryons (a)	Difference (a−b)	
Specimen I									
34 37	5.0 6.0	5.5	4.5	−1.0	17.0 16.0	16.5	16.5	0	*a*-1
26 27	5.0 6.0	5.5	5.5	0	19.0 16.0	17.5	21.0	3.5	*a*-2
26 4	5.0 20.5	12.8	16.0	3.2	19.0 56.0	37.5	22.0	−15.5	*b*-1
24 11	8.0 20.0	14.0	8.5	−5.5	27.0 41.0	34.0	23.5	−10.5	*b*-2
9 10	16.5 22.7	19.6	28.5	8.9	45.0 51.3	48.2	67.5	19.3	*c*-1
9 11	16.5 20.0	18.3	33.0	14.7	45.0 41.0	43.0	62.0	19.0	*c*-2
4 12	20.5 23.5	22.0	31.0	9.0	56.0 47.5	56.8	67.0	10.2	*c*-3
24 10	8.0 22.7	15.4	36.5	21.1	27.0 51.3	39.2	68.0	28.8	*b*-3
27 12	6.0 23.5	14.8	38.0	23.2	16.0 57.5	36.8	75.0	38.2	*b*-4
19 12	0 23.5	11.8	39.5	27.7	12.0 57.5	34.8	78.0	43.2	*b*-5
Specimen II									
6 20	12.5 28.5	20.5	31.0	10.5	37.0 67.0	52.0	68.5	16.5	*c*-4
6 3	12.5 32.5	22.5	31.0	8.5	37.0 74.0	55.5	67.0	11.5	*c*-5
22 6	12.5 12.5	12.5	36.5	24.0	33.5 37.0	35.25	68.5	33.2	*c*-6
14 1	13.0 33.5	23.3	39.5	16.2	40.5 65.5	53.0	72.0	19.0	*c*-7
5 3	6.5 32.5	19.3	49.0	29.7	13.0 74.0	43.5	90.0	46.5	*b*-6
Specimen III									
101 103	29.5 35.5	32.5	24.5	−8.0	71.5 76.0	73.8	77.0	3.2	*c*-8
Mean	17.4	16.9	29.5	11.4	49.4	42.5	58.9	16.6	
Standard deviation	11.01	6.86	13.21	11.69	21.15	14.46	23.82	15.20	
Coefficient of variability	63.27	40.59	44.78	102.54	42.81	34.05	40.31	109.64	

because of contaminations. Complete coverage of a 90-millimeter Petri dish in approximately three weeks time is recognized as the optimum growth rate of *M. americanus*.

In calculating the mean of the growth rate of the monokaryons, each growth rate measurement is used only once although the same monokaryon may appear more than once in the crossings. Thus in the 16

resulting dikaryons only 19 monokaryons are involved. In comparing a resulting dikaryon with its two monokaryon parents, the average measurement of the two monokaryons is used.

In both 7-day and 14-day measurements, the mean growth values and their standard deviations were larger in the dikaryophase than in the monokaryophase. However, the coefficient of variability was higher in the latter than in the former, especially in the 7-day record. The coefficient of variability of the differences between the two phases is exceedingly high and for both periods was over 100 percent. The correlation coefficient at seven days is 0.45 ± 0.21 and at fourteen days 0.42 ± 0.22. The correlation coefficient between the dikaryons and the means of their monokaryon parents was positive for both periods but significance was at the 10 percent level.

The results show that the growth of the dikaryophase is faster than the average of its monokaryotic components under the same conditions; or, in general, dikaryons grow faster than monokaryons. However, the reason for this is not known. Hybrid vigor has been well demonstrated in some species of higher plants, e.g., hybrid maize, and the condition of heterozygosity itself is the principal cause of it. Genetically, the dikaryophase is more complex than the monokaryophase and therefore it is possible that a somewhat analogous phenomenon also plays a part in the life of lower plants such as the fungi.

OIDIAL VARIANT

The powdery surface in a culture of oidium-bearing variants is usually smooth, occasionally rough. Aerial mycelium is lacking. Both smooth and rough surfaces may be seen in the same culture (FIG. 2, *E, F*). When this powdery trait predominates over the entire mat of the culture, the margin of the colony is usually uneven (FIG. 2, *E, F*). Short and weak aerial mycelium may be found at the periphery where it is less powdery. More often the oidial growth appears as a sector or sectors on both the monokaryotic and dikaryotic cultures. This occurs more frequently in the former than the latter. A few isolates from single basidiospores have developed directly into such oidial cultures. A reverse reaction of oidial cultures turning into mycelial cultures has not been observed.

Microscopic studies show that this powdery characteristic is associated with certain physiological factors of the organism. In contrast to the normal culture, it produces an abundance of rectangular, short, rod-shaped oidia. Crystals and particles are often present. Clamp-

connections usually are absent or difficult to locate if they occur in the dikaryotic cultures.

DISCUSSION

The variants described were of natural occurrence during the cultural characteristic studies. No artificial treatments were applied to induce variations. Diversities in the monokaryophase are greater than in the dikaryophase in so far as cultural characteristics and growth rate are concerned. This also holds true for the sequence of the appearance of variants under the same circumstances. Under usual laboratory conditions, dikaryon cultures remain fairly constant and are not difficult to identify, but some of the monosporous isolates look so different from the more typical ones that they might be easily mistaken for other species or contaminants. Recessive characters are more easily recognized in the monokaryophase. For example, certain characters such as color variations that might not show up in mass isolates would be more readily interpretable in cultures derived from combinations of two known monokaryons. Furthermore, it is improbable that monokaryotic mycelial wood rotters do not exist in nature just because reports on such findings are few, if any. The chances of their great diversity from the majority of the monokaryotic and dikaryotic cultures make their identification difficult. The knowledge of both phases is therefore requisite for a better recognition of them.

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STUDIES ON BAKER'S YEASTS OF EAST PAKISTAN

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The main diet of the people in East Pakistan is rice and this is deficient in vitamin B and proteins. Yeasts are a rich source of both vitamin B and proteins.

In order to meet this deficiency it was decided to isolate yeasts locally and to see if a suitable strain could be found or built up by hybridization which could synthesize all the members of vitamin B complex, and which possessed a high percentage of proteins. One of the sources from which yeasts were isolated was dough. The present paper constitutes a report of the studies conducted with baker's yeast obtained from four different districts of East Pakistan.

MEDIA AND METHODS

Media and methods employed in this investigation were the same as reported by Ahmad, Choudhury, and Ahmad in their studies on Toddy Yeast.

EXPERIMENTS AND RESULTS

Dough was obtained from four districts of East Pakistan, namely Rajshahi, Pabna, Dacca and Comilla. Thirty-six strains were isolated from Dacca, 18 from Pabna, 15 from Comilla and 10 from Rajshahi. These strains were tested for their different morphological and physiological characteristics and classified.

Seven isolates belonged to *Saccharomyces cerevisiae* Hansen, 26 to *Saccharomyces cerevisiae* var. *ellipsoideus* (Hansen) Dekker, 42 to *Saccharomyces carlsbergensis* Hansen, 2 to *Candida Guilliermondii* (Cast.) Lang. & Guerra, and 2 to *Rhodotorula glutinis* (Fres.) Harrison. The distribution of these strains in the different districts is shown in TABLE I.

All the strains of *Saccharomyces cerevisiae*, *S. cerevisiae* var. *ellipsoideus*, *S. carlsbergensis* and *Candida Guilliermondii* fermented dextrose, sucrose, raffinose, and galactose, but failed to ferment lactose. The two strains of *Rhodotorula glutinis* failed to ferment the above sugars as well as maltose.

TABLE I

DISTRIBUTION OF YEASTS IN DOUGH OBTAINED FROM RAJSHAHI, PABNA, DACCA, AND COMILLA

Name of locality	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* var. *ellipsoideus*	*Saccharomyces carlsbergensis*	*Candida Guilliermondii*	*Rhodotorula glutinis*
Rajshahi		8	2		
Pabna	3	8	7		
Dacca	4	10	18	2	2
Comilla			15		
Total	7	26	42	2	2

Vitamin synthesis tests showed that out of the 7 strains of *Saccharomyces cerevisiae*, 2 were autotrophic, 1 deficient for pantothenic acid, 1 deficient for biotin and inositol, 1 deficient for pantothenic acid and inositol, 1 deficient for pantothenic acid, biotin and pyridoxin, and

TABLE II

VITAMIN REQUIREMENTS OF 79 YEASTS ISOLATED FROM DOUGH

Designation of yeast	Section	No. of strains	Growth in synthetic media									
			Basal solution	Pantothenic-less	Biotin-less	Pyridoxin-less	Inositol-less	Niacin-less	Thiamin-less	Riboflavin-less	All vitamins	Total
Saccharomyces cerevisiae	a	2	+	+	+	+	+	+	+	+	+	
	b	1	−	−	+	+	+	+	+	+	+	
	c	1	−	+	−	+	−	+	+	+	+	
	d	1	−	−	+	+	−	+	+	+	+	7
	e	1	−	−	−	−	+	+	+	+	+	
	f	1	−	−	−	+	−	+	+	+	+	
Saccharomyces cerevisiae var. *ellipsoideus*	a	1	+	+	+	+	+	+	+	+	+	
	b	22	−	−	+	+	+	+	+	+	+	26
	c	2	−	−	−	+	+	+	+	+	+	
	d	1	−	−	−	+	+	−	−	−	+	
Saccharomyces carlsbergensis	a	15	+	+	+	+	+	+	+	+	+	
	b	6	−	−	+	+	+	+	+	+	+	
	c	7	−	−	−	+	+	+	+	+	+	
	d	2	−	−	+	+	−	+	+	+	+	
	e	1	−	−	+	−	+	+	+	+	+	
	f	1	−	−	−	−	+	+	+	+	+	
	g	2	−	−	+	−	−	+	+	+	+	42
	h	1	−	−	+	−	+	−	+	+	+	
	i	1	−	−	+	−	−	−	+	+	+	
	j	1	−	−	−	−	−	+	+	+	+	
	k	2	−	−	−	−	+	−	+	+	+	
	l	1	−	−	−	+	+	−	−	−	+	
	m	1	−	−	−	−	−	−	+	−	+	
	n	1	−	−	−	−	−	−	−	+	+	
Candida Guilliermondii		2	−	−	−	+	+	+	+	+	+	2
Rhodotorula glutinis		2	+	+	+	+	+	+	+	+	+	2
Grand total		79		52	22	12	11	8	5	3		

1 deficient for pantothenic acid, biotin and inositol (TABLE II). Of the 26 isolates belonging to *S. cerevisiae* var. *ellipsoideus*, 1 proved to be autotrophic, 22 deficient for pantothenic acid, 2 deficient for pantothenic acid and biotin, and 1 deficient for pantothenic acid, biotin, nicotinic acid, thiamin and riboflavin. Of the 42 isolates belonging to *Saccharomyces carlsbergensis*, 15 proved to be autotrophic, 6 deficient for pantothenic acid, 7 deficient for pantothenic acid and biotin, 2 deficient for pantothenic acid and inositol, 1 deficient for pantothenic acid and pyridoxin, 1 deficient for pantothenic acid, biotin and pyridoxin, 2 deficient for pantothenic acid, pyridoxin and inositol, 1 deficient for pantothenic acid, pyridoxin and nicotinic acid, 1 deficient for pantothenic acid, pyridoxin, inositol and nicotinic acid, 1 deficient for pantothenic acid, biotin, pyridoxin and inositol, 2 deficient for pantothenic acid, biotin, pyridoxin and nicotinic acid, 1 deficient for pantothenic acid, biotin, nicotinic acid, thiamin and riboflavin, 1 deficient for pantothenic acid, biotin, pyridoxin, inositol, nicotinic acid and riboflavin, and 1 deficient for pantothenic acid, pyridoxin, inositol, nicotinic acid and thiamin. The two isolates belonging to *Candida Guilliermondii* proved to be deficient for pantothenic acid and biotin, while the two isolates belonging to *Rhodotorula glutinis* proved to be autotrophic.

TABLE III

MATING REACTION AND SPORULATION CAPACITY OF SINGLE SPORE CULTURES FROM ONE ISOLATE BELONGING TO S. CEREVISIAE VAR. ELLIPSOIDEUS, AND 5 ISOLATES BELONGING TO S. CARLSBERGENSIS

Designation of species	Designation of strain	No. of single spore cultures tested	α		β		Neutral	
			Sporers	Non-sporers	Sporers	Non-sporers	Sporers	Non-sporers
S. cerevisiae var. *ellipsoideus*	Cx_2	21		12			9	
S. carlsbergensis	Cvi_5	19		11		8		
	Cv_2	13		8		5		
	B_3	15		7		8		
	$Ciii_2$	5		2		3		
	Ci_5	5		1		4		

FURTHER STUDIES ON 6 ISOLATES

One isolate belonging to *S. cerevisiae* var. *ellipsoideus* (Cx_2), and five isolates belong to *S. carlsbergensis* (Cvi_5, Cv_2, B_3, $Ciii_2$ and Ci_5) in which four-spored asci were common, were selected for further studies

of the mating system, and the homozygosity and heterozygosity of genes concerned in the fermentation of dextrose, sucrose, maltose, raffinose, galactose, α-methyl glucoside and the synthesis of various members of the vitamin B complex.

Twenty-six asci were dissected from Cx_2. Of 104 spores, only 44 germinated. In no case did all four spores of an ascus germinate. In 7 asci 3 spores germinated from each ascus and as best information with regard to segregations could be had from them, single spore cultures from these 7 asci only were tested for various characters. Of the five isolates belong to *S. carlsbergensis*, 18 asci were dissected from Cvi_5, 25 asci from Cv_2, 40 asci from B_3, 25 asci from $Ciii_3$ and 50 asci from Ci_5. Nineteen single spores germinated from Cvi_5, 13 from Cv_2, 15 from B_3, 5 from $Ciii_3$, and 5 from Ci_5. All these single spore cultures were tested for various characters.

Mating system. When the 21 single spore cultures from Cx_2 belonging to *Saccharomyces cerevisiae* var. *ellipsoideus* were tested for mating reaction, 12 gave an α [1] mating reaction and 9 gave no reaction. When all 21 cultures were tested for sporulation, the 12 single spore cultures which showed an α mating reaction failed to form spores whereas the 9 cultures which gave no mating reaction formed spores (TABLE III).

On testing single spore cultures from the five strains of *S. carlsbergensis* all of them were found to be bithallic [2] (heterothallic) as some single spore cultures from each of them proved to be α and some β

[1] The complementary strains of bipolar self-incompatible types have been designated as α and β by Ahmad (**1953b**), and they correspond to α and a of Lindegren (**1949**).

[2] Since heterothallism has come to be used both for separation of sexes in gametophytes as well as for the designation of complementary strains of monoecious self-incompatible types, Ahmad (**1953b**) redefined the terms homothallism and heterothallism and proposed new terminology as follows:

Homothallic: Primitive bisexual gametophytes in which no morphological differentiation of male and female gametes and gametangia has taken place.

Heterothallic: Primitive unisexual gametophytes in which the sexes are separated only physiologically and neither sex can be recognized as male or female for lack of morphological differentiation of gametes and gametangia.

Monoecious: Types in which recognizable male and female gametangia and/or gametes are borne by the same plant.

Dioecious: Types in which recognizable male gametangia and/or gametes are borne by one set of plants and female gametangia and/or gametes by the other set.

Monothallic: Types in which sexual reproduction proceeds normally in uninucleate single spore cultures.

Bithallic: Types in which the initiation and/or consummation of sexual reproduction is deferred in uninucleate single spore cultures till they come across the complementary strain.

(TABLE III). All single spore cultures from these five strains of *S. carlsbergensis* lacked the capacity to form spores.

Fermentation capacities. All 6 isolates, whose progenies have been analyzed, fermented dextrose, sucrose, maltose, raffinose and galactose, but failed to ferment lactose. Of the 6 strains, α-methyl glucoside was fermented only by Cx_2. When the 21 single spore cultures from Cx_2 belonging to *S. cerevisiae* var. *ellipsoideus* were tested, all of them fermented dextrose, sucrose and maltose but failed to ferment lactose. Thirteen single spore cultures fermented raffinose but 8 failed to ferment it; 15 single spore cultures fermented galactose but 6 did not ferment this sugar; likewise 11 single spore cultures fermented α-methyl glucoside but 10 failed to ferment it (TABLE IV).

TABLE IV

CAPACITIES OF SINGLE SPORE CULTURES FROM SIX STRAINS FOR FERMENTING VARIOUS SUGARS AND α-METHYL GLUCOSIDE

\+ Capacity to ferment within forty-eight hours present
− Capacity to ferment in forty-eight hours absent

Designation of species	Designation of strain	No. of single spore cultures tested	Capacity to ferment													
			Dextrose		Sucrose		Maltose		Raffinose		Galactose		Lactose		α-methyl glucoside	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−
S. cerevisiae var. *ellipsoideus*	Cx_2	21	21	0	21	0	21	0	13	8	15	6	0	21	11	10
S. carlsbergensis	Cvi_5	19	19	0	19	0	19	0	19	0	19	0	0	19	0	19
	Cv_2	13	13	0	13	0	13	0	13	0	8	5	0	13	0	13
	B_3	15	15	0	15	0	15	0	15	0	15	0	0	15	0	15
	$Ciii_3$	5	5	0	5	0	5	0	5	0	5	0	0	5	0	5
	Ci_5	5	5	0	5	0	5	0	5	0	5	0	0	5	0	5

All single spore cultures tested from the five strains of *S. carlsbergensis* (Cvi_5, Cv_2, B_3, $Ciii_3$ Ci_5) fermented dextrose, sucrose, maltose, and raffinose but failed to ferment lactose and α-methyl glucoside as did the parents (TABLE IV). Galactose was fermented by all single spore cultures from Cvi_5, B_3, $Ciii_3$, and Ci_5, but 5 single spore cultures out of 13 from Cv_2 failed to ferment it.

Vitamin synthesis capacities. With reference to vitamin synthesis, Cx_2 belongs to section (b) of *Saccharomyces cerevisiae* var. *ellipsoideus* (TABLE II); Cvi_5 and Cv_2 to section (a), B_3 and Ci_5 to section (b) and $Ciii_3$ to section (g) of *Saccharomyces carlsbergensis*. All 21 single spore cultures from the strain Cx_2 proved to be non-synthesizers of pantothenic acid and synthesizers of biotin, pyridoxin, inositol, nicotinic acid, and thiamin. One single spore culture out of 21 from Cx_2

failed to synthesize riboflavin (TABLE V). The 19 single spore cultures from Cvi_5 synthesized biotin, inositol, nicotinic acid, thiamin, and riboflavin. Unlike the parent, 4 single spore cultures out of 19 failed to synthesize pantothenic acid, and 4 cultures also failed to synthesize pyridoxin. All 13 single spore cultures from Cv_2 synthesized biotin, pyridoxin, nicotinic acid and riboflavin. Five single spore cultures from Cv_2 failed to synthesize pantothenic acid, 1 failed to synthesize inositol, and 5 failed to synthesize thiamin. All 15 single spore cultures from B_3 and all 5 single spore cultures from Ci_5 failed to synthesize pantothenic acid but synthesized the remaining 6 members of the vitamin B complex. All 5 single spore cultures from the strain $Ciii_3$ failed to synthesize pantothenic acid, pyridoxin, and inositol but synthesized

TABLE V

CAPACITIES OF SINGLE SPORE CULTURES FROM SIX STRAINS FOR SYNTHESIZING VARIOUS MEMBERS OF THE VITAMIN B COMPLEX

+ Synthesizer; − Non-synthesizer

Designation of species	Designation of strain	No. of single spore cultures tested	Growth in synthetic media													
			Pantothenic-less		Biotin-less		Pyridoxin-less		Inositol-less		Niacin-less		Thiamin-less		Riboflavin-less	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−
S. cerevisiae var. *ellipsoideus*	Cx_2	21	0	21	21	0	21	0	21	0	21	0	21	0	20	1
S. carlsbergensis	Cvi_5	19	15	4	19	0	15	4	19	0	19	0	19	0	19	0
	Cv_2	13	8	5	13	0	13	0	12	1	13	0	8	5	13	0
	B_3	15	0	15	15	0	15	0	15	0	15	0	15	0	15	0
	$Ciii_3$	5	0	5	3	2	0	5	0	5	3	2	5	0	3	2
	Ci_5	5	0	5	5	0	5	0	5	0	5	0	5	0	5	0

thiamin like the parent. Two single spore cultures from $Ciii_3$ failed to synthesize biotin, 2 failed to synthesize nicotinic acid and 2 failed to synthesize riboflavin.

DISCUSSION

Saccharomyces cerevisiae is most commonly associated with the raising of dough in the Western countries (Henrici *et al.* **1947**). *Candida Krusei* was, however, isolated from dough by Redaelli, Castelli and Ciferri (**1938**) and *Saccharomyces chevalieri* by Schaeffer [3] in prisoners' camps in Java during the last war.

Of the 79 yeasts isolated by the authors from dough, 42 belonged to *Saccharomyces carlsbergensis*, 26 to *Saccharomyces cerevisiae* var. *ellip-*

[3] Personal communication from Miss Genderen.

soideus, 7 to *Saccharomyces cerevisiae*, 2 to *Candida Guilliermondii* and 2 to *Rhodotorula glutinis*. As the Rhodotorulas failed to ferment dextrose, sucrose and maltose it is concluded that they are present merely as contaminants and are not concerned with the raising of the dough. Among the remaining 4 yeasts it is seen that *S. carlsbergensis* is the main baker's yeast in East Pakistan.

When the distribution of these yeasts from Comilla in the southeast to Rajshahi at a distance of about 150 miles in the northwest was examined, it was found that *S. carlsbergensis* alone was responsible for raising the dough at Comilla while most baker's yeast at Rajshahi proved to be *S. cerevisiae* var. *ellipsoideus* (TABLE I).

It may be recalled that Schaeffer isolated *S. chevalieri* from dough but the authors have failed to recover it so far from this source, although it is abundant in East Pakistan and has been isolated by Ahmad, Chaudhury, and Ahmad from toddy.

Of the 7 isolates belonging to *Saccharomyces cerevisiae*, 2 were autotrophes, 4 proved to be deficient for pantothenic acid, 3 deficient for biotin, 3 deficient for inositol and 1 deficient for pyridoxin. The data thus confirm the previous studies on the requirements of *S. cerevisiae* for pantothenic acid and biotin (Leonian and Lilly **1942**; Lindegren **1945**; Schultz and Atkin **1947**) and for inositol (Eastcott **1928**; Rogosa **1944**). Of the 26 isolates belonging to *S. cerevisiae* var. *ellipsoideus*, 1 was autotrophic, 25 deficient in pantothenic acid, 3 for biotin, 1 for nicotinic acid, thiamin and riboflavin. Of the 42 isolates belonging to *S. carlsbergensis* 15 were autotrophic, 27 proved deficient for pantothenic acid, 14 deficient for biotin, 11 deficient for pyridoxin, 8 deficient for inositol, 7 deficient for nicotinic acid, 4 deficient for thiamin and 2 deficient for riboflavin. The deficiency of 1 strain of *S. cerevisiae* var. *ellipsoideus* and 2 strains of *S. carlsbergensis* for riboflavin was unexpected, as no yeast so far has been reported to be deficient for this vitamin. Lack of synthesis of nicotinic acid by 1 strain of *S. cerevisiae* var. *ellipsoideus* and 7 isolates belonging to *S. carlsbergensis* contradicted the findings of Rogosa (**1943**) who maintains, "Yeasts which do not ferment lactose do not require an exogenous source of nicotinic acid for growth." The two Candidas were deficient for pantothenic acid and biotin, while the two Rhodotorulas were autotrophic. Thus, of the 79 isolates, 20 proved to be autotrophic, 58 deficient for pantothenic acid, 22 deficient for biotin, 12 deficient for pyridoxin, 11 deficient for inositol, 8 deficient for nicotinic acid, 5 deficient for thiamin and 3 deficient for riboflavin (TABLE II). It is thus seen that the deficiency for pantothenic acid is most widely spread among the yeasts examined by us.

When vitamin requirements of different yeast are examined, it is seen that yeasts belonging to different species and genera may have similar requirements, while yeasts belonging to the same species may have different requirements (TABLE II). Thus pantothenic acid- and biotin-requiring yeast can belong to *Saccharomyces cerevisiae* var. *ellipsoideus* (Section c, TABLE II), *S. carlsbergensis* (Section c) or *Candida Guilliermondii*. Schultz and Atkin (**1947**) have suggested that bios requirements may be used to differentiate yeasts belonging to a species into subspecies and varieties. The suggestion merits consideration, but it has been the experience of the authors that vitamin requirements of yeasts are not a very reliable characteristic as the same yeast occasionally gives a different performance. Schultz and Atkins have themselves found a lack of agreement in the bios requirements of 6 yeasts between their data and that of Burkholder. It, therefore, does not seem very safe, for the time being, to use bios requirements in classifying yeast.

The examination of mating reaction in the single spore cultures isolated from one strain of *S. cerevisiae* var. *ellipsoideus* (Cx_2) and five strains of *S. carlsbergensis* (Cv_2, Cvi_5, B_3, $Ciii_3$, and Ci_5) showed that all five strains of *S. carlsbergensis* were bithallic. Bithallism in *S. cerevisiae* var. *ellipsoideus* (Cx_2) was not clear cut as only α and neutral single spore cultures were isolated from the strain (TABLE III). The absence of β cultures is statistically significant. It is also seen that the 12 single spore cultures which gave an α mating reaction failed to form spores whereas the 9 single spore cultures which failed to give a mating reaction formed spores. This behavior of the single spore cultures from Cx_2 can best be accounted for by assuming that the β gene in it mutates rapidly to α and, on the creation of α cells in the β cultures, α and β cells fuse to give rise to neutral diploids; the diploid cells thus formed outgrow the original haploid cells, and hence, when these cultures are tested, they are found to be neutral and possess the capacity to form spores. This explanation on the basis of mutation of the mating type allele is more or less in keeping with the findings of Ahmad (**1953a**) in *S. cerevisiae* (Danish Baking Yeast).

When the single spore cultures from the 6 strains were tested for fermentation capacities, strain Cx_2 of *Saccharomyces cerevisiae* var. *ellipsoideus* was found to be homozygous for the genes concerned in the fermentation of dextrose, sucrose and maltose and heterozygous for raffinose, galactose and α-methyl glucoside fermentation genes (TABLE IV). The five strains of *S. carlsbergensis* analyzed were found to be homozygous for dextrose, sucrose, maltose and raffinose fermentation

genes. Four strains of *S. carlsbergensis* (Cvi_5, B_3, $Ciii_3$, and Ci_5) proved to be homozygous for galactose fermentation while one strain (Cv_2) proved to be heterozygous for the fermentation of this sugar. Although *S. carlsbergensis* is recorded in the literature (Lindegren **1949**, Lodder and Kreger-Van Rij **1952**) as a fermenter of galactose, yet the heterozygosity of strain Cv_2 shows that it is possible to have strains of this yeast which will fail to ferment galactose.

Strain Cx_2 of *S. cerevisiae* var. *ellipsoideus* proved to be homozygous for the synthesis of biotin, pyridoxin, thiamin, inositol and nicotinic acid (TABLE V). Out of 21 single spore cultures from this yeast one failed to synthesize riboflavin. This failure to synthesize riboflavin on the part of a single spore culture could follow from either the heterozygosity of more than one locus concerned in the synthesis of riboflavin or the mutation of the synthesizer allele to non-synthesizer. In view of the lack in these strains of mutants of other loci synthesizing various members of the vitamin B complex, the heterozygosity for riboflavin synthesis of another strain ($Ciii_3$), and the recovery of 3 riboflavinless strains, the former hypothesis seems to be nearer the truth. It appears that more than one locus is concerned in the synthesis of riboflavin in this strain and it is heterozygous at these loci.

Of the five strains of *S. carlsbergensis* analyzed, the strain Cvi_5 proved to be heterozygous for the synthesis of pantothenic acid and pyridoxin; strain Cv_2 proved to be heterozygous for pantothenic acid, inositol and thiamin synthesis and strain $Ciii_3$ showed heterozygosity for biotin, nicotinic acid and riboflavin synthesis. It must be stated that data from $Ciii_3$ and Ci_5 are too inadequate for definite inferences regarding homozygosity of genes concerned in fermentation of sugars and synthesis of various members of the vitamin B complex.

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SUMMARY

Seventy-nine strains of yeast have been isolated from dough from different districts of East Pakistan. Forty-two isolates belong to *Saccharomyces carlsbergensis*, twenty-six to *S. cerevisiae* var. *ellipsoideus*, seven to *S. cerevisiae*, two to *Candida Guilliermondii* and two to *Rhodotorula glutinis*. The two Rhodotorulas are considered to be contami-

nants. The isolation of *S. carlsbergensis* from dough is reported for the first time.

Nineteen isolates have proved to be autotrophic, fifty-eight deficient for pantothenic acid, twenty-two deficient for biotin, twelve deficient for pyridoxin, eleven deficient for inositol, eight deficient for nicotinic acid, five deficient for thiamin and three deficient for riboflavin.

Six strains of yeast have been studied further. The strain of *S. cerevisiae* var. *ellipsoideus* analyzed is heterozygous for the fermentation of raffinose, galactose and α-methyl glucoside and the synthesis of riboflavin. One strain of *S. carlsbergensis* is heterozygous for galactose fermentation. Heterozygosity for the synthesis of pantothenic acid and pyridoxin has been seen in one strain; for pantothenic acid, inositol and thiamin in another strain; and for biotin, nicotinic acid and riboflavin in a third strain of *S. carlsbergensis*.

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HYPHAL FUSION BETWEEN TRICHOPHYTON TONSURANS VARIANTS AS AN INDICATION OF SPECIES RELATIONSHIP[1]

CLAIRE L. TASCHDJIAN AND EMANUEL MUSKATBLIT[2]

(WITH 1 FIGURE)

Until recently the crateriform group of Trichophytons was considered to contain four species: *T. tonsurans* or *crateriforme*, *T. epilans* or *cerebriforme*, *T. Sabouraudii* or *acuminatum*, and *T. sulfureum* (Conant *et al.*, **1945**). In 1944 Carrión and Silva suggested inclusion of the three first-named species into one species, *T. tonsurans*, and retaining *T. sulfureum* as a second species in this group. Gonzalez Ochoa and Vasquez in 1945 proposed the establishment of a single species, *T. tonsurans*, with the varieties *epilans*, *Sabouraudii*, and *sulfureum*, and this proposal has now been generally adopted (Conant *et al.*, **1954**).

The clinical manifestations of *T. tonsurans* infection were recently summed up by Reiss (**1954**). All four variants of the species cause endothrix tinea capitis. The clinical condition may be that of Black Dot ringworm, often resembling diffuse alopecia, or acute inflammatory reactions and even kerion may be seen.

Cultures obtained from these conditions are extremely variable in their gross appearance. The colonies may present a heaped-up, acuminate center (var. *Sabouraudii*), or the center may be sunken, crateriform (var. *tonsurans*). The surface may be irregularly convoluted, cerebriform (var. *epilans*). The color of the aerial mycelium may be white, cream, primrose, rose, or sulfur yellow (var. *sulfureum*). The texture may be powdery or velvety. A red pigment may be present on the reverse of the colony.

Microscopically all these variants show close resemblance. They present pyriform or elongated, sometimes two-celled, microconidia ar-

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ranged *en thyrses* and sometimes *en grappes*. Macroconidia of the trichophytic type are seen occasionally (Georg, **1952**).

This resemblance in the microscopic morphology of cultures combined with the clinical picture of endothrix infection, as well as the great variability of the gross morphology of even monospore subcultures from one and the same strain, furnishes the main arguments for combining the four former species into the single species *T. tonsurans*.

Since these criteria are of a morphological and clinical rather than physiological nature, it was felt that additional physiological evidence might be of value in the reclassification of the *T. tonsurans* group. It is recognized that anastomosis will occur between mycelia of the same

TABLE I

T. TONSURANS STRAINS USED FOR HYPHAL FUSION EXPERIMENTS

Strain	Variety	Configuration	Color	Texture	Reverse pigment	Isolated and identified by*
1	sulfureum	convoluted	yellow	powdery	reddish brown	authors
2	tonsurans	convoluted	rose	powdery	reddish brown	Dr. F. Reiss
3	tonsurans	grooved	cream	powdery	deep red	authors
4	tonsurans	crateriform	white	velvety	orange	Dr. F. Reiss
5	tonsurans	convoluted	rose	powdery	deep red	authors
6	tonsurans	convoluted	cream	powdery	reddish brown	authors
7	Sabouraudii	acuminate	white	velvety	reddish brown	Dr. Gonzalez Ochoa
8	tonsurans	cerebriform	white	velvety	tan	Dr. F. Reiss
9	sulfureum	grooved	yellow	velvety	orange	Dr. F. Reiss
10	Sabouraudii	acuminate	white	powdery	tan	Dr. F. Reiss
11	tonsurans	crateriform	cream	powdery	tan	authors
12	tonsurans	crateriform	cream	powdery	tan	authors
13	tonsurans	convoluted	cream	powdery	orange brown	Dr. F. Reiss
14	tonsurans	convoluted	rose	powdery	deep purple	Dr. Gonzalez Ochoa
15	sulfureum (pleomorphic)	crateriform (slightly)	white	downy	orange	Dr. F. Reiss
16	tonsurans	crateriform	white	powdery	tan	Dr. Gonzalez Ochoa
17	tonsurans	crateriform	white	powdery	tan	Dr. Gonzalez Ochoa

* The authors wish to express their gratitude to Dr. F. Reiss, Department of Dermatology and Syphilology, New York University Post-Graduate Medical School, and to Dr. A. Gonzalez Ochoa, Institudo d'enfermidades tropicales, Mexico, for supplying them with the cultures listed above.

species, but not between mycelia of different species of fungi (Langeron and Vanbreuseghem, **1952**). While absence of fusion between two mycelia is not sufficient *per se* to warrant placing two organisms into different species, presence of fusion indicates that two strains do belong to one and the same species. Davidson, Dowding and Buller (**1932**), as well as Van Uden (**1951**), found anastomosis between different strains of *Microsporum audouini*, but not between *M. audouini* and *M. canis*. The occurrence of hyphal anastomosis between the four different types of *T. tonsurans* mentioned would therefore support further the view that all four are but variants of a single species.

MATERIAL AND METHOD

Seventeen strains of *T. tonsurans* were available for the experiments. The strains are enumerated and briefly described in TABLE I, while three representative types of the variants used are shown in FIG. 1.

Van Uden's method for demonstrating anastomosis between *Microsporum* strains did not give good results with *T. tonsurans*. We adapted

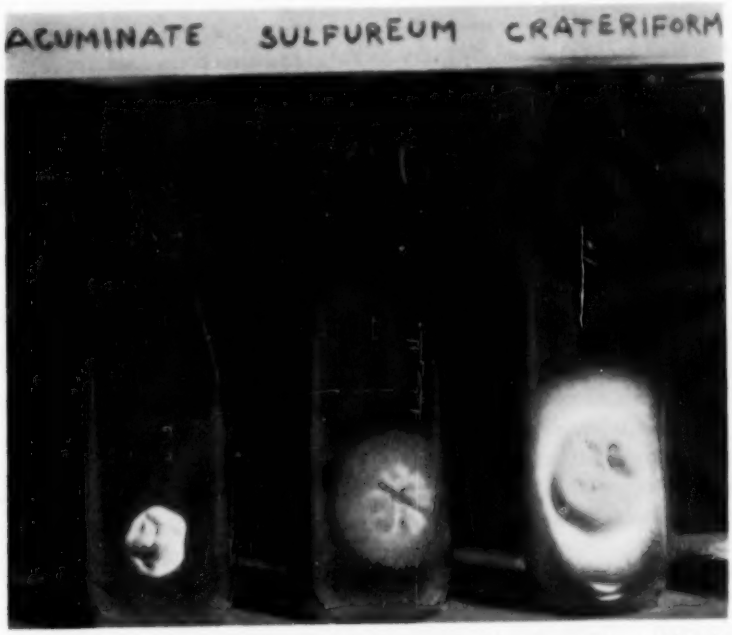

FIG. 1. Three variants of *Trichophyton tonsurans*, formerly considered as separate species: (left to right) *T. Sabouraudii* (*T. acuminatum*), *T. sulfureum*, *T. crateriforme*.

instead a method which we have found advantageous for growing fungi in slide cultures (Taschdjian, **1954**):

Flamed cover glasses, 22 mm², were dipped into melted culture medium and deposited on the surface of an agar plate. Sabouraud's medium containing 0.8% instead of 2% agar was used in order to increase the fluidity and transparency of the medium. Pinpoint inocula from two strains to be paired were placed circa 3 mm apart on the coated cover slip. The plates were sealed and incubated until the two

growing mycelia were seen to interlace under the low power lens of the microscope, usually after one week.

The cover glasses were then flooded with 95% ethanol and allowed to dry in a warm place. They were then stained with lactophenol-cotton-blue for 20 minutes. After removal of excess dye with filter paper, the cover slip cultures were inverted into a drop of pure glycerine on a clean slide and the preparations sealed after a suitable drying period.

The sealed slides were examined for anastomoses between the two mycelia. Only those were considered where the fusing hyphae could be traced back without difficulty to their respective "mother" mycelium.

RESULTS

Fusions were found to occur when each of two strains of the variety *sulfureum* were paired with each of 14 strains of the variety *crateriforme*, and with each of two strains of the variety *Sabouraudii*.

The two strains of the variety *Sabouraudii* fused with each of eight strains of the variety *crateriforme*.

Fusion was also obtained between a normal and a pleomorphic strain of the variety *sulfureum*, and between the pleomorphic strain and normal strains of the variety *crateriforme*.

The fusions observed were of the several types described by Koehler (**1930**). A short side branch produced from one hypha fused with a parallel hypha from the other strain which put forth a knob-like projection at the junction, resulting in an H-shape. Hyphae crossing at angles anastomosed by means of short lateral branches, forming the letter A. In some instances a lateral branch fused with a hypha some distance away.

End-to-end fusion of hyphae growing towards each other was not observed. There was no evidence of auto-antagonism.

CONCLUSIONS

The results of our experiments lend further validity to the reclassification of the crateriform Trichophytons into the single species *T. tonsurans*. The somewhat tedious procedure involved in hyphal fusion studies should not discourage workers from using this criterion to obtain information regarding other species of fungi whose systematic position is at present uncertain.

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GENERA OF MUCORALES WITH NOTES ON THEIR SYNONYMY

C. W. Hesseltine[1]

It is the purpose of this paper to present a short synopsis of the families and genera of the Mucorales. This can be justified, for members of the order are among the most commonly encountered and most economically important phycomycetous fungi. Furthermore, there is no recent comprehensive treatment of the families and genera published in English except that of Fitzpatrick (**7**) and Christenberry (**5**), who have followed earlier European treatments.

To make this contribution as useful as possible, the synonymy of the genera in the order has been given as completely as possible. Following the International Code of Botanical Nomenclature (**9**), the genera herein recognized are considered to be acceptable and are in general designated by those names in common use with the exception of the genus *Syzygites* (*Sporodinia*). In each case the synonyms are cited, as well as the type taxon, and an estimation of the number of species recognized in each genus. In the case of monotypic genera, therefore, the key is equivalent to that of a species key.

The keys and arrangement of genera into families are the conclusions drawn from more than ten years of interest and study of the order. The author has been fortunate to have seen or to have available material of practically all the genera and many of the species of this order. However, much of the arrangement of families is not new but rather a composite of the opinions of many earlier investigators. Neither is it implied that the following scheme is a final one, for it undoubtedly will need to be modified and enlarged as new relationships are established and new forms are found. The discovery of the perfect state in many cases will be of great value in clarifying uncertainties which now exist.

It is not within the scope of this paper to discuss the detailed problems of synonymy of the genera since this in turn is related to the question of individual species. It is hoped that this can be dealt with in later, more extensive papers on specific genera. Likewise, a considera-

[1] Contribution from the Northern Utilization Research Branch, Peoria, Illinois, one of the branches of the Agricultural Research Service, U. S. Department of Agriculture.

tion of the family Endogonaceae is purposely omitted, for the writer feels that he is too lacking in adequate, first-hand experience with the group to attempt even a general discussion of the forms belonging to it. A suggested treatment of the group can be found in Zycha (**17**).

The origin of the concept that the mucors constitute an order is not readily determined. It is true that Fries (**8**) had erected an order for the mucors but this was far from being correct in that certain genera which he included are obviously not Phycomycetes and, furthermore, the group was placed in the Hyphomycetes. The beginning of a real understanding of the Mucorales dates from 1873 when van Tieghem and Le Monnier (**15**) and later van Tieghem in 1875 and 1876 (**13, 14**) described a great number of forms. Van Tieghem envisioned these as belonging in a family Mucoraceae. This manner of treatment was essentially the same as used by Berlese and de Toni in Saccardo (**3**) with the exception that the subfamily Chaetocladieae had been added. Schroeter (**12**) treated the mucors as a suborder and later Fischer (**6**) gave us apparently a modern concept in his order Mucorinae, which embodied five families. Subsequent workers such as Zycha have closely followed this system and all have recognized the group as constituting an order.

As outlined below, the order would include Phycomycetes which have non-motile sporangiospores or conidia and a zygosporic sexual state, except as noted below. In the more common asexual state these forms are free living or, in some cases, parasitic upon other molds or fungi. The members of one family are usually found on higher plants but not on insects except as chance contaminants. Isolates of many of these forms are very rapid growing and often completely fill the petri dish with aerial mycelium.

The order is distinguished from the related order Entomophthorales by the nature of the zygospore wall, which is said to be formed from the modification of the gametangial wall in the Mucorales but not in the Entomophthorales. From a practical standpoint this is not of much help. Actually in the Entomophthorales conidia are present which often are discharged as conidia though later these may function as sporangia. They are often found on insects or other animals. In the Mucorales sporangia are the rule and, when conidia are formed, they are never forcibly discharged, never catenulate and are always borne directly on vesicles or on phialides upon swollen vesicle-like structures except possibly in certain forms as in the Mortierellaceae where 1-spored sporangia are known. The mycelium of the mucors is usually much more extensive than that of the Entomophthorales. A third related order, Zoopa-

gales, is distinguished because of the parasitic habit of its members on soil and aquatic animals with reproduction by means of conidia which may be in chains. They are attached to the host by haustoria-like organs.

MUCORALES

Mycelium branched, at first coenocytic, later commonly septate, extensive in development; aerial mycelium prominent, fertile, arising from substrate mycelium; asexual reproduction mainly by spores produced in sporangia which are always nonmotile, the latter in some cases accompanied by or replaced by conidia; conidia, when present, usually borne upon swellings on conidiophores, never catenulate; oidia and chlamydospores often present; sexual reproduction by means of thick-walled zygospores formed by fusion of equal or somewhat unequal coenogametangia; zygospores usually developed aerially, free or sometimes surrounded by a weft of mycelium; terrestrial, mostly saprophytic; sometimes parasitic on other members of the order or on higher plants but very rarely on insects.

Key to the families of the Mucorales modified from G. W. Martin's "Key to the Families of Fungi" in Ainsworth and Bisby's "A Dictionary of the Fungi" (1).

1. Sporocarp present, containing sporangia, zygospores or azygospores ..... ENDOGONACEAE
1. Sporocarp lacking 2
2. Sporangia all columellate and alike 3
2. Columellate sporangia present or absent; noncolumellate sporangia, sporangiola, merosporangia or conidia always present 4
3. Sporangial membrane thin, fugaceous; sporangiospores liberated by breaking up of sporangial wall; zygospores rough, suspensors usually not tong-like ..... MUCORACEAE
3. Sporangial wall densely cutinized above, entire sporangium violently discharged or detached as a whole from sporangiophore; zygospores smooth, suspensors tong-like ..... PILOBOLACEAE
4. Terminal sporangium columellate, multispored, or sometimes replaced by a sterile spine or with neither present; sporangiola (few or 1-spored) borne on branches of the same sporangiophore .... THAMNIDIACEAE
4. Columellate sporangia lacking (except in Choanephoraceae); imperfect stage represented by noncolumellate sporangia, sporangiola, merosporangia or conidia, or some combination of these structures 5
5. Asexual state represented by merosporangia or conidia borne upon phialides on a sporocladium 6
5. Merosporangia lacking; conidia not borne on phialides on a sporocladium 7

6. Merosporangia borne on swollen tips of sporangiophores, at first cylindrical, then forming a single row of sporangiospores, simulating a chain of conidia.....PIPTOCEPHALIDACEAE
6. Imperfect stage with only conidia; conidia borne on phialides which in turn are borne upon a sporocladium; sporocladia often on a branch or directly upon a conidiophore.....KICKXELLACEAE
7. Sporangiola or conidia borne on swollen tips (columellate sporangia also present in some genera); zygospores naked.....8
7. Sporangia, if present, without columellae; sporangiola and conidia, when present, borne singly, not on swollen tips of sporophores; zygospores imbedded in a thick hyphal matrix.....MORTIERELLACEAE
8. Genera forming conidia only; with zygospores formed as in *Mucor*.....CUNNINGHAMELLACEAE
8. Genera always possessing sporangia and sometimes conidia; zygospores not formed as in *Mucor*.....CHOANEPHORACEAE

MUCORACEAE Bonorden, Handbuch d. Allgemeinen Mycologie 121. 1851.

Mycelium nearly always rapid-growing and extensive; sporangiophores simple or branched; sporangia multispored, globose to pyriform, sometimes with an apophysis, columella always present, sporangia not deciduous nor violently discharged; conidia and true sporangiola absent; sporangial wall usually not cutinized; rhizoids and stolons sometimes present; zygospores with roughened walls, isogamous or heterogamous; suspensors smooth or with projections, not usually tong-like; saprophytic, never obligately parasitic.

Since much of the history of the family is associated with the order and follows in the discussion of the history of the succeeding families, no attempt will be made to trace its history.

The family is distinguished from the other families of the order by possessing only sporangia, though some sporangia may be considerably reduced in number of spores. Sporangia, which are always columellate, are never forcibly discharged and never possess both a cutinized wall and a subsporangial swelling. Sporangia, if cutinized, never with the sporangial wall rupturing at its junction with the columella. The sexual apparatus is of two basic types, one in which the suspensors are parallel to each other and the other, more rarely, in which they are tong-like with outgrowths, as seen in *Phycomyces*. All forms possessing conidia, merosporangia or sporangiola are excluded from the family.

Forms occurring in this family may be found in soil, on decaying plant material, on dung, on other fungi and in the air. They are almost universally present in any organic material which is in contact with the air.

In listing the genera and their synonyms, the accepted names are those in current usage except for the genus *Syzygites* which needs a word of explanation. The name *Syzygites* was first used by Ehrenberg (1818) for the zygosporic stage of this homothallic, monotypic genus. In a later paper (1820) Ehrenberg illustrated it with two plates showing the fungus growing on an agaric as well as depicting various stages of development of the zygospores. Ehrenberg's illustrations showed zygospores with large swollen suspensors which are in a straight line with the large zygospore. Also, the peculiar hyphae which grow from near the origin of the suspensors and are unique for the genus are clearly shown. Another figure showed a dichotomously branched stalk, which may be a very young sporangiophore or zygophore. There can be no doubt as to the identity of the form Ehrenberg was studying. Link, in 1824, had seen the sporangial stage of this organism and described it as *Sporodinia grandis*. Fries, in his treatment of the above forms in his "Systema," reduced *Sporodinia grandis* Link to synonymy with *Aspergillus maximus* but recognized Ehrenberg's genus and maintained the species name *Syzygites megalocarpus*. Immediately following the treatment of *Syzygites* in this work, Fries placed a genus *Azygites*, found on agarics, which, according to Fischer (**6**), probably belongs here, having been based upon material with azygospores.

Waalroth, a year after Fries' work, used the genus name *Sporodinia* Link apparently for the sporangial stage of two species which he described, hence validating the name *Sporodinia*. It was not until the work of the Tulasnes in 1866 (**16**), of de Bary in 1864 (**2**) and Brefeld in 1881 (**4**) that the true connection between the zygosporic and the sporangial stages was demonstrated. The Tulasnes and de Bary both used the name *Syzygites megalocarpus* in preference to *Sporodinia grandis*.

The common use of the name *Sporodinia grandis* probably goes back to van Tieghem's use of it in 1875 and later to its use by Schroeter and by Fischer. Zycha (**17**) and Naumov (**11**) each have used *Sporodinia*, both in direct violation of the International Code of Botanical Nomenclature.

The type genus of the family is *Mucor*.

KEY TO THE GENERA OF THE MUCORACEAE

1. Sporangia flask-shaped with a distinct spherical venter and a long neck.....SAKSENAEA
   Saksena, Mycologia **45**: 434. 1953.
   Type species: *S. vasiformis* Saksena.
   One species known.

1. Sporangia not flask-shaped.....2
2. All sporangia pyriform and with a definite apophysis; sporangia borne from stolons but typically not opposite the rhizoids.....ABSIDIA
van Tieghem, Ann. Sci. Nat. VI. **4**: 350. 1876.
Type species: *A. reflexa* van Tieghem.
Syn.: *Tieghemella* Berlese and de Toni. In Saccardo's Syll. Fung. **7**: 215. 1888. *Mycocladus* Beauverie, Ann. Univ. Lyon **3**: 162. 1900. *Lichtheimia* Vuillemin, Bull. Soc. Mycol. Fr. **19**: 126: 1903. *Proabsidia* Vuillemin, Bull. Soc. Mycol. Fr. **19**: 126. 1903. *Pseudoabsidia* Bainier, Bull. Soc. Mycol. Fr. **19**: 155. 1903. *Protoabsidia* Naumov, Clés des Mucorinées, 76. 1939.
Eighteen or 19 species are known.
2. Spherical sporangia on upright sporangiophores and pyriform sporangia borne circinately on flaccid sporangiophores.....PIRELLA
Bainier, Ann. Sci. Nat. VI. **15**: 84. 1883. Étude sur les Mucorinées (Thèse, Paris), 83. 1882.
Type species: *P. circinans* Bainier.
One species known.
2. All sporangia spherical; if stolons present, then sporangiophores opposite the rhizoids.....3
3. Sporangia with a definite apophysis.....4
3. Sporangia without an apophysis.....5
4. Sporangiophores formed mostly on stolons opposite the rhizoids.....RHIZOPUS
Ehrenberg ex Corda, Icon. Fung. **2**: 20. 1838. Ehrenberg, Nova Acta Acad. Leop. **10** (1): 198. 1820.
Type species: *R. stolonifer* (Ehrenberg ex Fries) Vuillemin. Rev. Mycol. **24**: 54. 1902.
Syn.: *Ascophora* Tode ex Fries, Syst. Myc. **3**: 309. (*pro parte*) 1832. *Philophora* Wallroth, Flora Kryptog. Germ. **4** (*2*): 332. 1833.
Exact number is uncertain but many species reported.
4. Sporangiophores not on stolons.....SPINELLUS
van Tieghem, Ann. Sci. Nat. VI. **1**: 66. 1875.
Type species: *S. fusiger* (Link ex Fries) van Tieghem.
Five or six species known.
5. Sporangiophores usually on stolons with rhizoids.....ACTINOMUCOR
Schostakowitsch. Ber. Deut. Bot. Ges. **16**: 155. 1898.
Confusion exists as to the type species though Schostakowitsch used *A. repens*. This will be clarified in a future publication on the genus.
Syn.: *Glomerula* Bainier, Bull. Soc. Mycol. Fr. **19**: 154. 1903.
One species known.
5. Sporangiophores not on stolons.....6
6. Sporangiophores regularly dichotomously branched; sporangia all of the same size; homothallic.....SYZYGITES
Ehrenberg ex Fries, Syst. Myc. **3**: 329. 1832.
Ehrenberg, Sylvae Mycol. Berol. 25. 1818 and Verh. Gesellsch. naturf. Freunde Berlin **1**: 98. 1820.

Type species: *S. megalocarpus* Ehrenberg ex Fries.
Syn.: *Sporodinia* Link, Spec. Plant. **6** (1): 94. 1824. *Azygites* Fries, Syst. Orb. Veg. 364. 1825. *Azygites* Fries ex Fries, Syst. Myc. **3**: 330. 1832. *Sporodinia* Link ex Wallroth, Flora Krypt. Germ. **4** (2): 317. 1833.
One species is known.

6. Sporangiophores at least partly dichotomously branched; large terminal sporangia and smaller sporangia on branches; homothallic.....DICRANOPHORA
Schroeter, Jahresb. schles. Gesell. vaterl. Kultur **64**: 184. 1886.
Type species: *D. fulva* Schroeter.
One species known.

6. Sporangiophores irregularly divided or simple.....7

7. Sporangiophores branched with all the branches bearing sporangia circinately.....CIRCINELLA
van Tieghem & Le Monnier, Ann. Sci. Nat. V. **17**: 298. 1873.
Type species: *C. umbellata* van Tieghem and Le Monnier.
Syn.: *Circinumbella* van Tieghem and Le Monnier, Compt. Rend. Acad. Sci. **74**: 999. 1872. (*Nomen provisiorum.*)
Eight or nine species known.

7. No sporangia or not all the sporangia on branches borne circinately.....8

8. Large sporangiophores over 80 mm high with a metallic luster, unbranched; suspensors with branched, finger-like projections....PHYCOMYCES
Kunze ex Fries, Syst. Myc. **3**: 308. 1832. Kunze, Mycol. Hefte **2**: 113. 1823.
Type species: *P. nitens* Kunze ex Fries.
Four species are known.

8. Sporangiophores shorter, or, if 80 mm, then branched and without metallic luster; suspensors usually unadorned.....9

9. Never parasitic upon other members of the Mucorales; suspensors unadorned.....10

9. Gall-forming parasites upon other Mucorales; suspensors with finger-like projections.....PARASITELLA
Bainier, Bull. Soc. Mycol. Fr. **19**: 153. 1903.
Type species: *P. parasitica* (Bainier) Sydow, Ann. Mycol. **1**: 371. 1903.
One or possibly two species known.

10. Zygospores constantly on short side branches of the sporangiophores; homothallic; heterogamous.....ZYGORHYNCHUS
Vuillemin, Bull. Soc. Mycol. Fr. **19**: 116. 1903.
Type species: *Z. heterogamus* Vuillemin.
Six or seven species known.

10. Zygospores on separate hyphae, seldom upon sporangiophores; mostly heterothallic; isogamous.....MUCOR
Micheli ex Fries, Syst. Myc. **3**: 317. 1832.
Micheli, Nova Plantarum Genera **1**: 215. 1729.
Type species: *M. caninus* Persoon ex Fries.

Syn.: *Ascophora* Tode, Fungi Mecklenb. sel. **1**: 13. 1790. *Hydrophora* Tode, Fungi Mecklenb. sel. **2**: 5. 1791. *Ascophora* Tode ex Fries, Syst. Myc. **3**: 309 (*pro parte*). 1832. *Hydrophora* Tode ex Fries, Syst. Myc. **3**: 313. 1832. *Chionyphe* Theinemann, Nova Acta Acad. Leop. **19**: 19. 1839. *Calyptromyces* H. Karsten, Bot. Zeit. **7**: 365. 1849. *Pleurocystis* Bonorden, Handb. Allge. Mykol. 124. 1851. *Scitovszkya* Schulzer. v. Muggenburg, Verh. Zool.-Bot. Ges. Wien **16**: 36. 1866. *Chlamydomucor* Brefeld, Unters. Gesam. Mykol. **8**: 223. 1889. *Amylomyces* Calmette, Ann. Inst. Pasteur **6**: 611. 1892. *Ascidophora* Reich.? (*fide* Saccardo, Syll. Fung. **15**: 48. 1901.)
About 60 species are known.

PILOBOLACEAE Corda, Icon. Fung. **1**: 22. 1837.

Sporangiophores simple or branched, phototrophic, often with adhering droplets, with or without a subsporangial swelling, often arising from a trophocyst; sporangia colored, typically black, cutinized, multispored, sometimes forcibly discharged, sporangial wall persistent with lower portion thin and lighter in color, containing many sporangiospores and a mucilaginous material, the latter swelling and causing the sporangial wall to break at the base; zygospores nearly smooth, spherical or globose, produced between two parallel or tong-like suspensors at the surface of the substrate; coprophilous.

The family Pilobolaceae is based upon the genus *Pilobolus* which was first recognized as a distinct genus by Tode. Up until the time of Fries this genus was considered to be closely related to the genus *Mucor*, being placed in it or near it. Fries, after including *Pilobolus* in the Gasteromycetes in 1823, transferred it to his order "II Mucorini" in the class Hyphomycetes which included such genera as *Mucor, Syzygites, Phycomyces, Ascophora*, etc. Corda in 1837 introduced the name Pilobolidae and indicated that it was of family rank. At first he included in this family *Pilobolus* and *Chordostylum;* later in 1842, he added the genera *Pycnopodium* and *Caulogaster*. Corda's figures of the genus *Chordostylum* Tode show an object which is probably not a fungus, and if it is, it certainly does not belong in the Zygomycetes. The genus *Pycnopodium* was created by Corda for his *Pilobolus lentigerus*. The fourth genus *Caulogaster* was erected by Corda but he gave no figures in his "Icones Fungorum" and but a short description; however, according to Ainsworth and Bisby (**1**), Tulasne stated that this genus was based upon insect eggs.

The genus *Pilaira* was erected by van Tieghem and it, as well as *Pilobolus*, was placed in the tribe Pilobolées. Schroeter treated these

two genera as belonging in a subfamily Pilobolei under the family Mucoracei. Fischer in 1892 followed Schroeter and maintained these as members of this subfamily. Later monographers have treated them as distinct families except that Zycha placed both under the family Mucoraceae without even recognizing them as sufficiently distinct to make a subfamily.

To the writer *Pilobolus* and *Pilaira* appear to constitute a well-defined family. The zygospores of both genera are similar in the manner of their origin, location on the mycelium and in the character of their walls, which are almost smooth. They are, on the other hand, decidedly different from the zygospores of *Mucor, Absidia,* and *Phycomyces* of the family Mucoraceae, for the latter are produced usually aerially, have verrucose walls and are sometimes adorned with accessory outgrowths from the suspensors. In addition, the cutinization of the sporangial wall and the manner of breaking open of the wall are characters which indicate close affinities between *Pilobolus* and *Pilaira* and which further seem to justify setting these genera apart in a separate family.

The type genus is *Pilobolus.*

1. Not discharging sporangia violently and without a subsporangial swelling ..... PILAIRA
   van Tieghem, Ann. Sci. Nat. VI. **1**: 51. 1875.
   Type species: *P. anomala* (Cesati) Schroeter (in Cohn's Kryptog.-Flora v. Schlesien **3** (*2*): 211. 1886).
   Four or five species are known.

1. Discharging sporangia violently and with a subsporangial swelling .... PILOBOLUS
   Tode ex Fries, Syst. Myc. **2**: 308. 1823.
   Tode, Schrift. d. Berl. Gesell. Naturf. Freunde **5**: 46. 1784.
   Syn.: *Pycnopodium* Corda, Icon. Fung. **5**: 18. 1842.
   Type species: *P. crystallinus* Tode ex Fries.
   Nine species are known.

THAMNIDIACEAE Brefeld, Bot. Unters. Schimmelpilze **4**: 161. 1881.

Mycelium *Mucor*-like; sporangiophores dichotomously or irregularly branched or with branches in a whorl-like arrangement, usually bearing both sporangia and sporangiola upon the same sporangiophores, light-colored, bearing a globose sporangium or a spine terminally, but in one genus with neither a terminal spine nor sporangium; branches of the sporangiophores ending in sporangiola or sharp-pointed spines; sporangiola usually with persistent walls, borne on straight or circinate stalks, globose or pyriform, one to few-spored, often without a well-

developed columella; sporangia multispored, deliquescing, with columellae; sporangiospores hyaline, smooth-walled; zygospores formed between two unappendaged suspensors, not enclosed by a hyphal envelope; usually saprophytic and often preferring rather low temperatures.

The members of this family have been variously treated. Brefeld in 1881 erected two families which contained members of this family as here construed. These he called the Thamnidieen and the Chaetocladiaceen. Schroeter recognized Chaetocladiaceae but not Thamnidiaceae. Fischer's treatment is similar to Schroeter's but Lendner used the two families of Brefeld. Naumov, on the other hand, reduced both Thamnidiaceae and Chaetocladiaceae to a single tribe, Thamnidieae, in the family Mucoraceae. Zycha, however, placed both Chaetocladiaceae and Thamnidiaceae together as the Thamnidiaceae and within this family placed the last four genera treated below and, in addition, the genus *Dicranophora*. Zycha's grouping seems to be much more natural than those previously used.

What one may think of as the basic situation in the family is seen in *Thamnidium*. Here there is a sporangium at the tip of the main axis of the sporangiophore while globose sporangiola are borne at the ends of all the branches which are repeatedly dichotomously branched. From this situation one advances to that found in *Chaetostylum* where some branches instead of ending in sporangiola end in terminal spines but a typical terminal sporangium is formed at the end of the sporangiophores. *Chaetocladium* represents the end of the development in which the sporangiola have been reduced to one-spored structures and the large *Mucor*-like sporangium is completely missing. A fourth genus, *Helicostylum*, is closely related to *Thamnidium* but has circinately borne sporangiola which are usually pyriform in shape. A recently described genus *Cokeromyces* possesses only circinately borne sporangiola, and has typical *Mucor*-like zygospores but fails to have any large sporangia or sterile spines.

The genera of this family are unlike those of the Choanephoraceae in that the sporangiola are formed upon the same sporangiophores as the sporangia. The sporangiola of *Chaetocladium*, although one-celled, definitely possess a sporangial wall which can be observed readily, whereas the conidia of the Choanephoraceae and Cunninghamellaceae do not show a comparable structure, at least not by ordinary methods of study.

The type genus is *Thamnidium*.

The following key is given for the genera of the Thamnidiaceae.

1. Sporangiola never borne circinately; sporangiola never pyriform.....3
1. Sporangiola borne circinately; these typically pyriform or globose.....2
2. Terminal sporangia never present; homothallic.....COKEROMYCES
Shanor, Mycologia **42**: 272. 1950.
Type species: *C. recurvatus* Poitras, *l.c.*
One species known.
2. Terminal sporangia present; heterothallic.....HELICOSTYLUM
Corda, Icon. Fung. **5**: 18 and 55. 1842.
Type species: *H. elegans* Corda.
Syn.: *Haynaldia* Schulzer v. Muggenburg, Verh. Zool.-Bot. Ges. Wien **16**: 37. 1866. Also *Thamnidium* of some authors.
Five or six species reported.
3. No sterile spines at the ends of branches.....THAMNIDIUM
Link ex Wallroth, Flora Kryptog. Germ. **4** (*2*): 324. 1833.
Link, Gesellsch. naturf. Freunde, Berlin Mag. **3**: 31. 1809.
Type species: *T. elegans* Link ex Wallroth.
Syn.: ?*Melidium* Eschweiler, De fructificatione generis Rhizomorphae commentato 33. 1822. *Melidium* Eschweiler ex Fries, Syst. Myc. **3**: 330. 1832.
Three species known.
3. Sterile spines at the ends of branches.....4
4. Several sporangiospores to a sporangiolum.....CHAETOSTYLUM
van Tieghem & Le Monnier, Ann. Sci. Nat. V. **17**: 328. 1873.
Type species: *C. fresenii* van Tieghem & Le Monnier.
Syn.: *Bulbothamnidium* Klein, Verh. Zool.-Bot. Ges. Wien **20**: 557. 1870.
One or two species known.
4. Single-spored sporangiola only.....CHAETOCLADIUM
Fresenius, Beiträge zur Mykologie, p. 97. 1863.
Type species: *C. jonesii* (Berkeley & Broome) Fresenius.
Two species are known.

PIPTOCEPHALIDACEAE Brefeld, Bot. Unters. Schimmelpilze **4**: 161. 1881.

Mycelium hyaline, delicate, branched; sporangiophores branched or unbranched; modified sporangia (merosporangia) cylindrical or elongate, relatively few-spored; sporangiospores arranged in a single row; sporangial wall deliquescing; zygospores formed between tong-like suspensors, in one genus said to be *Mucor*-like, never surrounded by mycelium; mostly obligate parasites upon other Mucorales.

De Bary discovered the genus *Piptocephalis* and later van Tieghem and Le Monnier added the genus *Syncephalis*. Van Tieghem later described a third genus, *Dispira*, and included these three genera within his tribe Syncephalidées. Brefeld created the family shortly afterwards and it was accepted by Schroeter who also erected a fourth genus, *Syncephalastrum*. Fischer, however, changed the name of the family to Cephalidaceae which has been used by Lendner and Zycha. Zycha included in this family not only the four genera given above but also

added *Coemansia, Kickxella* and *Spinalia.* Naumov divided the family into two families with Syncephalastraceae containing only *Syncephalastrum* and the family Cephalidaceae containing *Syncephalis, Piptocephalis* and *Dispira.* The family name Piptocephalidaceae is retained here because of priority and because of appropriateness.

The family's one characteristic in common is the production of few-spored sporangia with the sporangiospores arranged in a nearly linear manner. There is a tendency also for several genera, *Syncephalis* and *Piptocephalis,* to be obligate parasites on other fungi, particularly on other Mucorales.

The type genus is *Piptocephalis* de Bary. The three recognized genera may be separated as follows:

1. Saprophytic or facultatively parasitic on other Mucorales; sporangiophores not dichotomously branched.....SYNCEPHALASTRUM
   Schroeter, in Cohn's Kryptog.-Flora v. Schlesien **3** (*2*): 217. 1886.
   Type species: *S. racemosum* Schroeter.
   One to three species are known.
1. Obligate parasites usually upon other Mucorales (*Dispira* is a possible exception); sporangiophores unbranched or if branched then branched dichotomously.....2
2. Sporangiophores dichotomously branched always several times.....3
2. Sporangiophores unbranched (in one species *S. furcata* once-branched).....SYNCEPHALIS
   van Tieghem & Le Monnier, Ann. Sci. Nat. V. **17**: 372. 1873.
   Type species: *S. cornu* van Tieghem & LeMonnier.
   Twenty-five to 30 species have been reported.
3. Some of the branches prolonged into sterile prongs; sporangiferous heads not deciduous.....DISPIRA
   van Tieghem, Ann. Sci. Nat. VI. **1**: 160. 1875.
   Type species: *D. cornuta* van Tieghem.
   One species is known.
3. All the branches terminating in sporangiferous heads, the latter deciduous.....PIPTOCEPHALIS
   de Bary, Abh. Senckenb. Natf. Ges. **5**: 356. 1865.
   Type species: *P. freseniana* de Bary.
   Twelve to 15 species are known.

KICKXELLACEAE Linder, Farlowia **1**: 56. 1943.

Mycelium hyaline or light-colored; sporangia absent; conidiophores simple or branched, always with sporocladia; sporocladia somewhat inflated, septate or non-septate, frequently tapering to one to three terminal cells and bearing phialides upon its upper or lower surface; phialides simple, hyaline, ovoid to elongate-ellipsoid; conidia borne singly at the ends of the phialides, one-celled, sometimes pseudoseptate, hyaline

to yellow, elongate-ellipsoid to needle-shaped, sometimes with a capsule at one end; zygospores as in the Piptocephalidaceae.

This family was created by Linder who monographed the family. The treatment presented here follows closely Linder's ideas of the genera and the manner in which they can be recognized.

Coemans described the genus *Kickxella* in 1861 as a fungus which had sporocladia (special branches of the conidiophore which bear phialides on which single conidia are borne) arranged in a whorl at the end of a simple conidiophore. Along with this fungus he also mentioned sporangia and cleistothecia. The resulting confusion is described in detail by Linder. Coemans described a second genus, *Martensella*, with conidia on the upper side of sporocladia which were borne singly along the conidiophore, and van Tieghem and Le Monnier added a third genus, *Coemansia*, with the conidia on the lower side. There is some doubt as to whether these genera may actually be distinct. Recently, a fourth genus, *Linderina*, was described in which the sporocladia were not septate and not clearly pedicellate. The sporocladia were ovoid or dome-shaped, and not boat-shaped as in the other genera.

The earlier genera have been variously treated. Coemans considered *Martensella* to be a Hyphomycete. On the other hand, van Tieghem and Le Monnier believed that *Coemansia* was a member of the Mucorales. Recent treatments, such as Zycha's, recognized *Coemansia* and *Kickxella* as members of the Piptocephalidaceae while Naumov only mentioned them in his appendix to his treatment of the Mucorales.

The family is readily recognized by the production of sporocladia which bear conidia and by the absence of sporangia. The zygospores are believed to be similar to those of the Piptocephalidaceae. Members of this family are found in soil, dung, and on other fungi.

The type genus is *Kickxella* Coemans.

A key to the genera is as follows:

1. Sporocladia in a whorl at the apex of the conidiophore; conidia pseudoseptate.....KICKXELLA
   Coemans, Bull. Soc. Roy. Bot. Belgique **1**: 155. 1862.
   Type species: *K. alabastrina* Coemans.
   Syn.: *Coronella* Crouan & Crouan. Florule d. Finistre 12. 1867. *Coemansiella* Saccardo, Syll. Fung. **2**: 815. 1883. *Ibid.* **4**: 55. 1886.
   A single species known.

1. Sporocladia not in a whorl, borne pleuracrogenous on conidiophores; conidia not pseudoseptate.....2

2. Sporocladia nonseptate, ovoid or dome-shaped, but never elongated...LINDERINA
   Raper & Fennell, Am. Jour. Bot. **39**: 81. 1952.
   Type species: *L. pennispora* Raper & Fennell.
   A single species known.

2. Sporocladia always elongate and septate.....3
3. Sporocladia always bearing conidia on the lower surface.....COEMANSIA van Tieghem & Le Monnier, Am. Sci. Nat. V. **17**: 392. 1873.
Type species: *C. reversa* van Tieghem & Le Monnier.
Approximately 16 species are known.
3. Sporocladia bearing conidia on the upper surface.....MARTENSELLA Coemans, Bull. Acad. Roy. Belgique II. **15**: 292, 540. 1863.
Type species: *M. pectinata* Coemans.
Two species are known.

MORTIERELLACEAE Fischer, In Rabenhorst's Kryptog.-Flora v. Deut. Oest. u. Schweiz **1** (4): 268. 1892.

Mycelium coenocytic, later septate, branched; sporangiophores simple or branched, hyaline, forming sporangia terminally; sporangia from one- to many-spored, globose; columellae absent; zygospores enclosed in a more or less compact covering of mycelium.

Coemans described *Mortierella* as the first-known member of the family. Van Tieghem in his system of classification placed this genus under the tribe *Mortierellées* of the family Mucoraceae. Berlese and de Toni considered *Mortierella* and *Herpocladium* Schroeter to be members of the subfamily Mortierelleae while Fischer elevated this subfamily to family rank. Schroeter and subsequent monographers have accepted this arrangement while Thaxter added two additional genera, *Haplosporangium* and *Dissophora.*

Very recently Novotelnova has described a fourth genus, *Naumoviella,* as follows: "Aerial mycelium abundant, hyaline. Sporangiophores not very different from the mycelium, and producing branches laterally, branched two to three times at right angles. Columellae absent. Sporangia few-spored." The Latin description of the single species, *N. nivea,* and the illustrations would indicate that he was dealing only with another species of *Mortierella* and, therefore, it is being treated as a synonym of this genus. Even more recently Ribaldi described still another genus, *Gongronella,* in this family. For a discussion of this form see below.

Mortierellaceae is recognized because the sporangium completely lacks a columella and because the zygospores are covered by a weft of mycelium. Unfortunately this latter characteristic has been seen only in a few species of *Mortierella* and not at all in *Haplosporangium* or *Dissophora.*

The type genus is *Mortierella.* The genera may be separated as follows:

1. Sporangia with one or two sporangiospores.....HAPLOSPORANGIUM
   Thaxter, Bot. Gaz. **58**: 362. 1914.
   Type species: *H. bisporale* Thaxter.
   Approximately six species known.
1. Sporangia with several sporangiospores.....2
2. Sporangiophores arising from the ordinary vegetative mycelium...MORTIERELLA
   Coeman, Bull. Acad. Roy. Sci. Belg. II. **15**: 288, 536. 1863.
   Type species: *M. polycephala* Coemans.
   Syn.: *Carnoya* Dewevre, Grevillea **22**: 4. 1893. and Bull. Soc. Belg. Microsc. **21**: 36. 1894. *Naumoviella* Novotelnova, Not. Syst. Crypt. Inst. Bot. Acad. Sci. URSS **6**: 155. 1950.
   Many species known.
2. Sporangiophores arising in progression on special fertile hyphae of indeterminate growth.....DISSOPHORA
   Thaxter, Bot. Gaz. **58**: 361. 1914.
   Type species: *D. decumbens* Thaxter.
   Only two species reported.

DOUBTFUL GENERA

1. HERPOCLADIUM Schroeter, in Cohn's Kryptog.-Flora v. Schlesien **3** (2): 213. 1886.
   Syn.: *Herpocladiella* Schroeter, in Engler and Prantl's Nat. Pflanzenfamilien **1** (1): 130. 1893.

This genus was erected by Schroeter for one species, *H. circinans*, which has never since been seen. Later Schroeter changed the generic name to *Herpocladiella* because a subgenus *Herpocladium* had already been used for another plant (Jungermannieae).

2. GONGRONELLA Ribaldi, Riv. Biol. Gen. N. S. **44**: 164. 1952.

The description and figures show a fungus closely related to, if not identical with, *Absidia butleri* except that the columella, though present, is greatly reduced. The measurements of the apophysis, sporangia and sporangiophores are all within the range of this *Absidia* species. *Gongronella urceolifera* was isolated from soil just as *A. butleri* always is. The color of the colonies and mode of branching are similar to *A. butleri*. Ribaldi considered that the genus belonged in the family Mortierellaceae but it has not been possible to secure a culture for comparative study.

CUNNINGHAMELLACEAE Naumov, Clés des Mucorinées, p. 20. 1939.

Mycelium coenocytic, later septate, branched; conidiophores branched or unbranched, bearing only conidia upon terminal swellings; sporangia and sporangiola never formed; conidia borne singly, never catenulate, often echinulate, hyaline, one-celled, never appearing as one-spored sporangiola; zygospores *Mucor*-like, with lateral suspensors, verrucose.

This family was proposed by Naumov to consist of those genera in

the Choanephoraceae which bore only conidia. He, therefore, separated *Cunninghamella, Sigmoideomyces,* and *Thamnocephalis* from the genera *Choanephora* and *Blakeslea.* The last two genera both possess sporangia and in addition sporangiola or conidia.

Matruchot, who proposed the first genus in this family, *Cunninghamella,* believed that it belonged in the Mucorales near *Choanephora.* This belief was soon confirmed by the discovery that species were heterothallic and that zygospores were produced when opposite strains were mated. Lendner (**10**), in his systematic treatment of the genus, placed it in the family Chaetocladiaceae. But later treatments, such as those by Zycha and Fitzpatrick, have placed *Cunninghamella* with *Choanephora* and *Blakeslea.* Zycha, in addition to these three genera, placed the genera *Thamnocephalis, Sigmoideomyces,* and *Rhopalomyces* Corda in the family Choanephoraceae. The inclusion of *Rhopalomyces* in the Cunninghamellaceae does not seem warranted at this time for it is very probable that many of the species within it are not Phycomycetes. In addition to Naumov's three genera, the genus *Mycotypha* Fenner also seems to belong here. Although zygospores are not yet known, Fenner, as well as the writer, believed it to be a zygomycete. On the other hand, the genus *Sigmoideomyces* (see below) is treated here as a very doubtful member of the family.

The family is distinguished by having only conidia produced in the asexual state; the conidia are borne upon variously shaped swollen vesicles. The zygospores, where known, are verrucose, borne in a ladder-like fashion and in all respects are like those of *Mucor.* The zygospores of the Choanephoraceae, on the contrary, are produced between tong-like suspensors and the zygospore surface is nearly smooth.

The type genus is *Cunninghamella.*

The following key is given to the genera of the Cunninghamellaceae:

1. Vesicles upon which the conidia are borne cattail-like, hence extremely elongate and slender.....MYCOTYPHA
   Fenner, Mycologia **24**: 187. 1932.
   Type species: *M. microspora* Fenner.
   One species is known.

1. Vesicles globose or short ovoid.....2

2. Conidiophores mostly ramose, with a vesicular swelling at the apex of each branch: conidiophores rarely simple and terminated by a capitate enlargement.....CUNNINGHAMELLA
   Matruchot, Ann. Mycol. **1**: 46. 1903.
   Type species: *C. echinulata* (Thaxter) Thaxter (Rhodora **5**: 98. 1903).

Syn.: *Actinocephalum* Saito, Bot. Mag. Tokyo **19**: 36. 1905. *Saitomyces* Ricker, Jour. of Mycol. **12**: 61. 1906. *Muratella* Bainier & Sartory, Bull. Soc. Mycol. Fr. **29**: 129. 1913.
Six species are known.

2. Conidiophores having both sterile branches and branches ending in swollen fertile vesicles; vesicles spherical, borne on oppositely formed branches.....THAMNOCEPHALIS
Blakelee, Bot. Gaz. **40**: 165. 1906.
Type species: *T. quadrupedata* Blakeslee.
One species is known.

DOUBTFUL OR EXCLUDED GENERA

1. SIGMOIDEOMYCES Thaxter, Bot. Gaz. **16**: 22. 1891.

This genus has been placed in the Mucorales by both Naumov and Zycha. Although it may prove to be a member of the Mucorales it is at present exceedingly doubtful. The writer is of the opinion that when the perfect state of this fungus is found it will not prove to be a member of the Mucorales.

2. PRACHTFLORELLA Matruchot, Ann. Mycol. **1**: 56. 1903.

This name was proposed in the same paper as was the genus *Cunninghamella*. It was apparently based on the species *Gonatobotrys microspora* Rivolta which in turn cannot be recognized. It should be considered only as a provisional name.

CHOANEPHORACEAE Fitzpatrick, Lower Fungi 258. 1930. Emend. Naumov, Clés des Mucorinées 97. 1939.

Mycelium hyaline, much-branched, rapidly growing; sporangiola, conidia and sporangia always upon separate stalks; sporangiophores bearing many-spored sporangia or few-spored sporangiola, both with persistent walls; conidiophores divided, bearing dark-colored conidia that are often striate, sporangia columellate, often borne circinately; sporangiospores usually with bristle-like structures at each end, brown-colored, often striate; zygospores smooth or nearly so, borne between two closely appressed suspensors; typically growing on higher plants.

Blakeslea and *Choanephora*, which constitute this family, have been known for a long time, the former having been described by Thaxter in 1914 and the latter by Currey in 1873. Although it was recognized that these genera were closely related and hence placed next to each other, it was not until Naumov excluded the genus *Cunninghamella* from the family that the family assumed its present form. Fitzpatrick had considered the family to be composed of *Blakeslea*, *Choanephora* and *Cunninghamella*. Zycha, on the other hand, had used the family in a much broader sense to include *Rhopalomyces*, *Cunninghamella*, *Thamnocephalis* and *Sigmoideomyces*.

It would seem that the two genera presently recognized are closely related to each other but distinct from the other families of the order by having the conidia or sporangiola on fruiting stalks distinct from the sporangiophores, by possessing zygospores of a distinct type, by being short-lived in pure culture and by growing on higher plants.

Two other generic names have been applied to *Choanephora*. Currey had originally named it *Cunninghamia* but discovered in the same year that this name had already been used for a genus in the family Pinaceae. Hence the new generic name, *Choanephora*, had to be proposed. The genus *Choanephorella* was proposed by Vuillemin for the species *Rhopalomyces cucurbitarum*, but he failed to give a description or a figure of the fungus. Neither did he make any reference to where the species *R. cucurbitarum* had been published nor who had proposed this name. *Choanephorella* must be considered as a *nomen nudum*.

The type genus is *Choanephora*. The two genera may be readily separated as follows:

1. Conidia absent but sporangiola present.....BLAKESLEA
   Thaxter, Bot. Gaz. **58**: 353. 1914.
   Type species: *B. trispora* Thaxter.
   A single species is known.

1. Conidia present (one species *C. persicaria* with none) but sporangiola never present.....CHOANEPHORA
   Currey, J. Linn. Soc. Bot. **13**: 576. 1873.
   Type species: *C. infundibulifera* (Currey) Saccardo (Syll. Fung. **9**: 339. 1891).
   Syn.: *Cunninghamia* Currey, J. Linn. Soc. Bot. **13**: 334. 1873. (Obligate synonym.) *Choanephorella* Vuillemin, Bull. Soc. Mycol. Fr. **20**: 28. 1904. (*Nomen nudum.*)
   Four species are known.

DOUBTFUL OR EXCLUDED GENERA OF THE ORDER

1. ACROCYSTIS Ellis & Halsted, Bull. N. J. Agric. Stat. **76**: 20. 1890.

Ellis & Halsted imperfectly described this genus growing on sweet potatoes. The description is too incomplete to allow the genus to be recognized.

2. AZYGOZYGUM Chesters, Trans. Brit. Mycol. Soc. **18**: 199. 1933.

The single species, *A. chlamydosporum*, was believed by Chesters to belong in the Zygomycetes near *Mortierella*. This species, which was found in association with roots of *Antirrhium majus* near Birmingham, England, possessed chlamydospores and zygospore-like structures, but no sporangia or conidia were found.

3. DICOCCUM Corda, in Sturm's Deut. Flora, Pilze **2**: 117. 1829. Also Icon. Fung. **2**: 6. 1838.

Zycha placed this genus in the order, recognizing the single species *D. asperum*, which he considered to belong here because he believed the dark, rough spores were

azygospores. After examination of a culture of this fungus it appears to the writer better left in the Fungi Imperfecti.

4. DIMARGARIS van Tieghem, Ann. Sci. Nat. VI. **1**: 154. 1875.

Believed to be a form very similar to the genus *Dispira* and Zycha treated it as a synonym of this genus.

5. HILDEBRANDIELLA Naumov, Mat. Myc. and Phytopath. **1**: 61. 1917.

Syn.: *Syzigites echinocarpa* Hildebrand, Jahrb. f. wiss. Bot. **6**: 277. 1863.

This genus erected by Naumov is based on *H. echinocarpa* which was originally described by Hildebrand in 1867 as *Syzygites echinocarpus*. It is doubtful if Naumov saw any material which Hildebrand had studied. From the figures given by Hildebrand it would appear to be only *S. megalocarpus*. At most the genus is so ill-defined that it must not be recognized.

6. MASSARTIA de Wildeman, Soc. Belge Microsc. **21**: 27. 1897.

The sporangial stage was not seen. Smooth-walled zygospores were produced between tong-like suspensors and the zygospores were found in masses of mycelium. The one species, *M. javanica*, was found in the mucus of terrestrial algae from Java.

7. PERICYSTIS Betts, Ann. Bot. **26**: 798. 1912.

This fungus certainly is not a mucoraceous fungus but probably an Ascomycete.

8. RHIZOMUCOR Lucet & Costantin, Rev. Gen. Bot. **12**: 81. 1900.

This mold is definitely a member of the Mucorales. Zycha believed it to be *Actinomucor*. The discoverers thought it to be a form intermediate between *Rhizopus* and *Mucor*.

9. SPINALIA Vuillemin, Bull. Soc. Mycol. Fr. **20**: 32. 1904.

This genus is undoubtedly a member of the Mucorales. It possesses vesicles upon which are borne sterigma-like structures which then produce conidia at their ends.

However, both structures apparently break off as spores. Whether these spores are conidia or merosporangia is in doubt. The fungus, should it be rediscovered, needs more study of the development of the fruiting heads before it can be placed precisely in the correct family.

SUMMARY

A key is presented for the determination of the families and genera of the Mucorales. Histories of the arrangement of the genera in the nine families recognized are outlined. The generic synonymy, type species and approximate number of species present in each genus are given.

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rangement of families and genera, as well as the keys, are derived from the work done on the author's Ph.D. thesis at the University of Wisconsin, Madison, Wisconsin.

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ADDITIONAL SPECIES OF ZOOPAGACEAE SUBSISTING ON RHIZOPODS AND EELWORMS

CHARLES DRECHSLER[1]

(WITH 9 FIGURES)

The 8 fungi herein described as new members of the Zoopagaceae, and also the related form of uncertain specific identity that is included for consideration, were observed in Petri plate cultures that had been planted with pinches of slowly decaying vegetable detritus. In such cultures zoopagaceous fungi are, as a rule, found only sparingly intermixed with large quantities of much coarser forms. Consequently, they often are not clearly revealed even in a living state, and on immersion in mounting fluids usually become quite indiscernible. Except for a few of the more robust species it seems unlikely that dried cultures or permanent microscope mounts of the Zoopagaceae will prove very helpful in supplying information supplementary to published descriptive accounts. To remedy in some measure the lack of durable material as well as of pure cultures suitable for continued propagation, figures of the reproductive or vegetative bodies most distinctive of the separate species may with advantage be given in greater than customary numbers —in numbers preferably large enough to allow random selection of the bodies concerned. The approximately random assortments of conidia illustrated in the figures of *Acaulopage bicornis, A. longicornis,* and *A. dactylophora* are accordingly intended to display, much like material in microscope mounts, not only the usual moderate variations observable in the very distinctive asexual spores formed by these species but also, even if to a lesser extent, the more pronounced and more infrequent variations.

Random assortments of asexual reproductive bodies are likewise shown in the figures of the 2 amoeba-capturing species described as *Cystopage ellipsospora* and *C. sphaerospora.* The morphological difference thereby disclosed might, however, hardly suffice to separate the 2 fungi if it were not correlated with difference in widths of mycelial

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hyphae; for the smooth, unappendaged, globose chlamydospores here constituting the sole products of asexual reproduction are to an unfortunate degree lacking in distinctive detail. Similar difficulty in the delimitation of species is met in nearly all material referable to *Cystopage*, as neither sameness nor otherness can be readily determined from the meagerly expressive chlamydospores prevalent in the genus. Despite this difficulty it seems advisable to make a beginning in the description of amoeba-capturing members of *Cystopage* since they manifestly are no less efficacious than the more familiar conidial Zoopagaceae in their destruction of animal life, besides being of equally ubiquitous occurrence in decaying plant residues and of equally wide geographical distribution. Moreover, by making better known the outward similarity of the chlamydospores to the globose sporangia of many *Pythium* species it is hoped that the likelihood of confusion between the 2 types of asexual reproductive bodies may be reduced—opportunity for such confusion often coming about when species of *Cystopage* develop in Petri plate cultures prepared for the isolation of parasitic oomycetes from decaying roots and stems.

Acaulopage dasyspora sp. nov.

Mycelium exiguum sed saepe late extentum; hyphis mycelii continuis, incoloratis, parce ramosis, plerumque 0.9–1.3 μ crassis, ad amoebas inhaerentibus, animalia ita capientibus, pelliculam cujusque captivi perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio in aliquot (circa 3) ramulis plerumque circiter 20 μ longis et 1.2 μ latis constante; ramis fertilibus vulgo procumbentibus sed forsitan quandoque ascendentibus, basi plerumque circa 1 μ crassis sed interdum usque 2 μ crassis, apice unum conidium ferentibus; conidio incolorato, globoso, multis (60–100) prominentibus vestito, sine prominentibus plerumque 8–13 μ in diametro; prominentibus vulgo digitiformibus vel clavaeformibus, plerumque 1.5–1.9 μ longis, basi circa 0.5 μ latis, sursum interdum aliquid dilatatis vel lobosis itaque 0.5–1 μ latis.

Amoebas saepe 15–25 μ latas capiens consumensque habitat in radicibus *Phaseoli vulgaris* putrescentibus prope Fort Lauderdale, Florida, etiam in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium scanty yet often extensive; hyphae continuous, colorless, sparingly branched, mostly 0.9–1.3 μ wide, capturing amoebae through adhesion, penetrating the pellicle of each captive and then intruding a bush-like haustorium consisting of a few (about 3) assimilative branches often approximately 20 μ long and 1.2 μ wide; sporiferous branches commonly procumbent but probably in some instances rising into the air, most often about 1 μ wide throughout but sometimes up to 2 μ wide proximally, bearing terminally a single conidium; conidium colorless, globose, beset with many (60–100) protuberances, without the pro-

tuberance usually 8–13 μ in diameter; the protuberances finger-shaped or club-shaped, usually 1.5–1.9 μ long, about 0.5 μ wide at the base, 0.5–1 μ wide at the tip which often is expanded or bilobate.

Capturing and consuming amoebae often 15–25 μ wide, it occurs in decaying bean (*Phaseolus vulgaris* L.) roots near Fort Lauderdale, Florida, and also in decaying plant residues near Laplace, Louisiana.

Acaulopage dasyspora developed in several maize-meal agar plate cultures used in the isolation of *Pythium spinosum* Sawada from some bean seedlings which W. D. Moore kindly supplied late in January, 1953, as being illustrative of a root rot then occurring in southern Florida. It also came to light in a few maize-meal agar plate cultures that after being overgrown with mycelium of *Pythium ultimum* Trow had been further planted with small quantities of vegetable detritus collected in southern Louisiana on December 20, 1952. To its scanty mycelium small amoebae (FIG. 1, *A, a, b; B, a–c; C, a, b; D, a; E, a*), in many instances largely depleted of their protoplasm, were found attached at irregular intervals. The few assimilative branches making up the haustorium (FIG. 1, *A, a; C, b*) visible in animals undergoing expropriation of their contents differed little in width from the mycelial filaments.

Although asexual reproduction in the fungus began rather early, the formation of conidia seemed to proceed slowly and sparingly. Usually the young conidium appeared as a small swelling (FIG. 1, *B, d, e; C, c*) at the tip of a short branch given off nearly at a right angle to the parent hypha. However, in at least one instance a similar globose swelling was observed developing in a virtually sessile position (FIG. 1, *F, a*). For some time each swelling would continue to grow larger without any modification of its smooth spherical contour (FIG. 1, *C, d, e*), but in the final stages of expansion it would put forth many narrow protuberances from all portions of its surface except from a small proximal area (FIG. 1, *D, b; E, b; G*). Often the protuberances remained cylindrical (FIG. 1, *N, a*), but often, again, they would widen out distally like a pestle (FIG. 1, *N, b*) or would terminate in 2 divergent lobes (FIG. 1, *N, c, d*). In mature conidia the protuberances appeared empty. Usually the conidium was found delimited by a septum laid down 0.5 to 2.5 μ from the spherical contour, so that a stipe-like basal part could often be recognized when the spore was viewed laterally (FIG. 1, *H–J*) though not when it was viewed from above (FIG. 1, *K–M*).

With respect to its most distinctive feature, the abundant ornamentation of its conidia, *Acaulopage dasyspora* bears closest resemblance to my *A. hystricospora* (**9**).

Acaulopage dactylophora sp. nov.

Mycelium parcum sed saepe late passum; hyphis mycelii incoloratis, continuis, parce ramosis, 0.7–0.8 μ crassis; conidiis incoloratis, plerumque aliquid enormiter complanato-ellipsoideis, saepius bilobis vel trilobis, basi vulgo aliquantum umbonatis et interdum pedicello vacuo praeditis, sed deorsum ex magna parte levibus, sursum multis (10–30) prominentibus vacuis praeditis, sine prominentibus vulgo 7–9 μ longis et 9–14 μ latis—pedicello conidiorum 0.5–2 μ longo et circa 0.7 μ crasso, prominentibus conidiorum digitiformibus, 1.5–3.5 μ longis, 0.6–0.8 μ crassis.

Habitat in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium scanty yet often extensive; vegetative hyphae colorless, aseptate, meagerly branched, 0.7–0.8 μ wide; conidia colorless, usually flattened ellipsoidal in lateral view, often bilobate or trilobate in upper view, on the lower side generally smooth though often umbonate at the base where an empty pedicel is sometimes attached, on the lateral and upper surfaces beset with many (10–30) empty protuberances, exclusive of these protuberances commonly 7–9 μ long and 9–14 μ wide—the conidial pedicel 0.5–2 μ long and about 0.7 μ wide, the conidial protuberances finger-shaped, 1.5–3.5 μ long and 0.6–0.8 μ wide.

Occurring in decaying plant detritus near Laplace, Louisiana.

Acaulopage dactylophora appeared in several maize-meal agar plate cultures which after being overgrown by *Pythium ultimum* had been further planted with small quantities of decaying vegetable materials collected in southern Louisiana on December 20, 1952. In all of these cultures it was found intermingled with other minute members of the Zoopagaceae, so that its delicate mycelial hyphae (FIG. 2, *A*, *a*, *b*) could not usually be distinguished with certainty after portions of them had become evacuated to supply protoplasm for the formation of conidia. From the ambiguous material it was not possible to make out clearly which of the several protozoans found captured was being utilized as prey by the present species.

The scanty mycelium gave rise on the surface of the agar substratum to sparsely scattered conidia of varied design. Viewed laterally many of the asexual spores appeared broadly turnip-shaped (FIG. 2, *B*, *a–d*, *f*) though in others the generally flattened-ellipsoidal vertical profile of the living cell was modified strongly by sinuses and angular projections (FIG. 2, *B*, *e*; *g–i*). In many instances conidia viewed from above (FIG. 2, *B*, *j–t*) or below (FIG. 2, *B*, *u–z*) presented a somewhat bilobate (FIG. 2, *B*, *j*, *l*, *m*, *s*, *t*, *v*, *w*, *y*, *z*) or trilobate (FIG. 2, *B*, *k*, *u*, *x*) profile suggestive of desmids, but in other instances the horizontal profile of the living cell was irregular or unsymmetrical (FIG. 2, *B*, *n–r*). A pronouncedly bristling appearance was generally characteristic of the co-

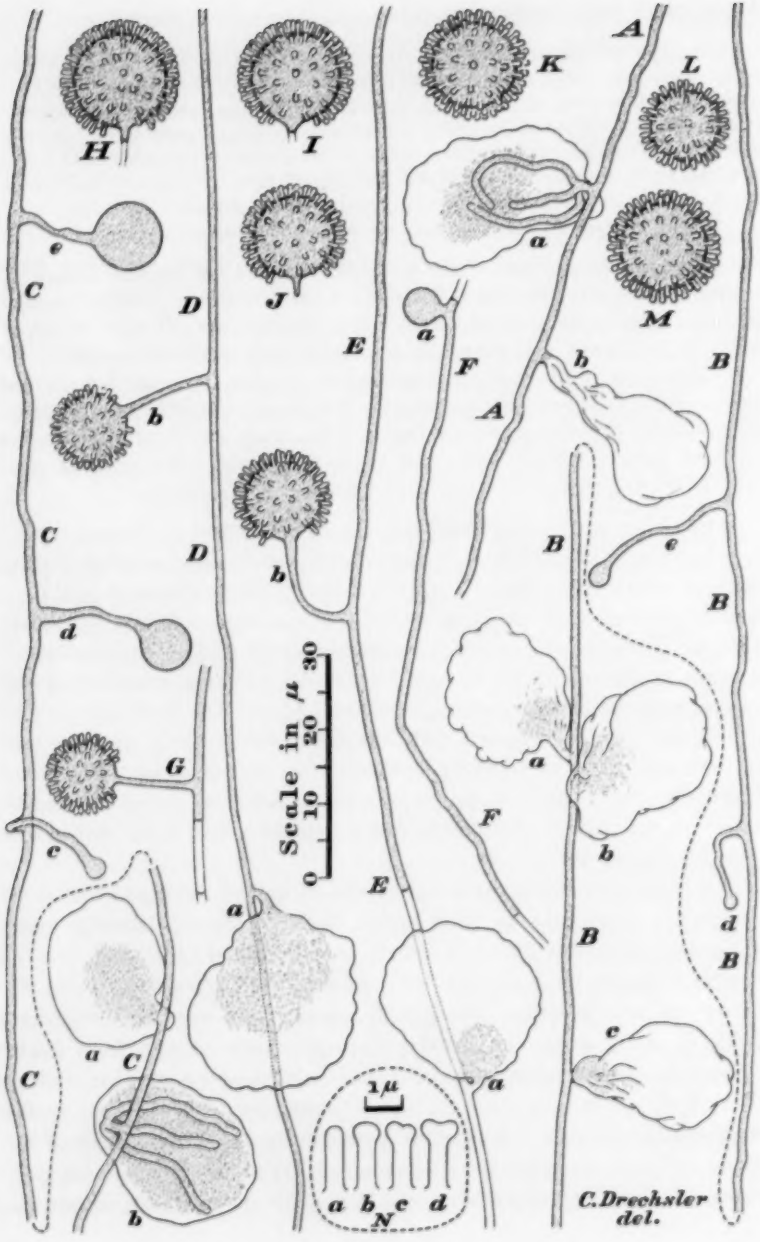

FIG. 1.

nidia, though the empty membranous protuberances giving them such appearance were considerably less numerous and less uniformly distributed than the corresponding protuberances in *Acaulopage dasyspora*. The empty protuberances in *A. dactylophora*, moreover, seemed always of unmodified digitate shape, never being found expanded at the tip. Nevertheless, the obvious similarities of conidial ornamentation shown in *A. dactylophora* must be held to relate the species closely to *A. dasyspora* and *A. hystricospora*.

Acaulopage bicornis sp. nov.

Mycelium exiguum sed saepe se late pandens; hyphis mycelii incoloratis, continuis, parce ramosis, circa 1 μ crassis; conidiis sparsim oriundis, incoloratis vel aliquantulum fumidis, plus minusve ovoideis sed in apice appendice vacua praeditis, cellula eorum viva plerumque 9–20 μ longa et 5–12 μ lata; appendice interdum tubulo 5–10 μ longo et 1–1.8 μ lato constante, saepissime basi bifurca itaque in 2 ejusmodi tubulis consistente, sed quandoque alterutro tubulo vel ambobus tubulis semel raro bis bifurcis.

Habitat in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium scanty yet sometimes extensive, composed of aseptate, colorless, meagerly branched, filamentous hyphae measuring about 1 μ in width; conidia formed sparsely on the surface of the substratum, colorless or of slightly smoky tinge, ovoid, mostly 5–12 μ wide and 9–20 μ long exclusive of the empty membranous appendage attached at the tip —this appendage sometimes consisting of a single closed tube 5–10 μ long and 1–1.8 μ wide, but much more often bifurcating proximally into 2 such tubes, and sometimes, again, further dichotomizing once or twice in either or in both of the tubes resulting from the proximal bifurcation.

Occurring in decaying plant remains near Laplace, Louisiana.

FIG. 1. *Acaulopage dasyspora* obtained from Louisiana material; parts *A–M* drawn at a uniform magnification with the aid of a camera lucida, × 1060. *A*, Portion of hypha with 2 captured amoebae, *a* and *b*; a haustorium of 3 assimilative branches is shown within amoeba *a*, while amoeba *b* is represented only by an empty pellicle. *B*, Portion of hypha with 3 captured amoebae, *a–c*, and 2 young conidiiferous branches, *d* and *e*; each amoeba is represented by a virtually empty pellicle. *C*, Portion of hypha with 2 captured amoebae, *a* and *b*, and 3 conidiiferous branches, *c–e*; a haustorium is shown within amoeba *b*; the branches *c–e* show the terminal conidium in an early stage, a half-grown stage, and a nearly full-grown stage, respectively. *D*, *E*, Portions of hypha, each with a captured amoeba, *a*, and a conidiiferous branch, *b*. *F*, Short hyphal segment with a young conidium developing in a nearly sessile position. *G*, Short portion of hypha with a branch bearing an almost fully developed conidium. *H–J*, Fully developed conidia in lateral view. *K–M*, Fully developed conidia in top view. *N*, Conidial protuberances, *a–d*, showing differences in distal conformation; approximately × 5300. (Owing to lack of space *B* and *C* are each shown in 2 parts whose proper connection is indicated by a broken line.)

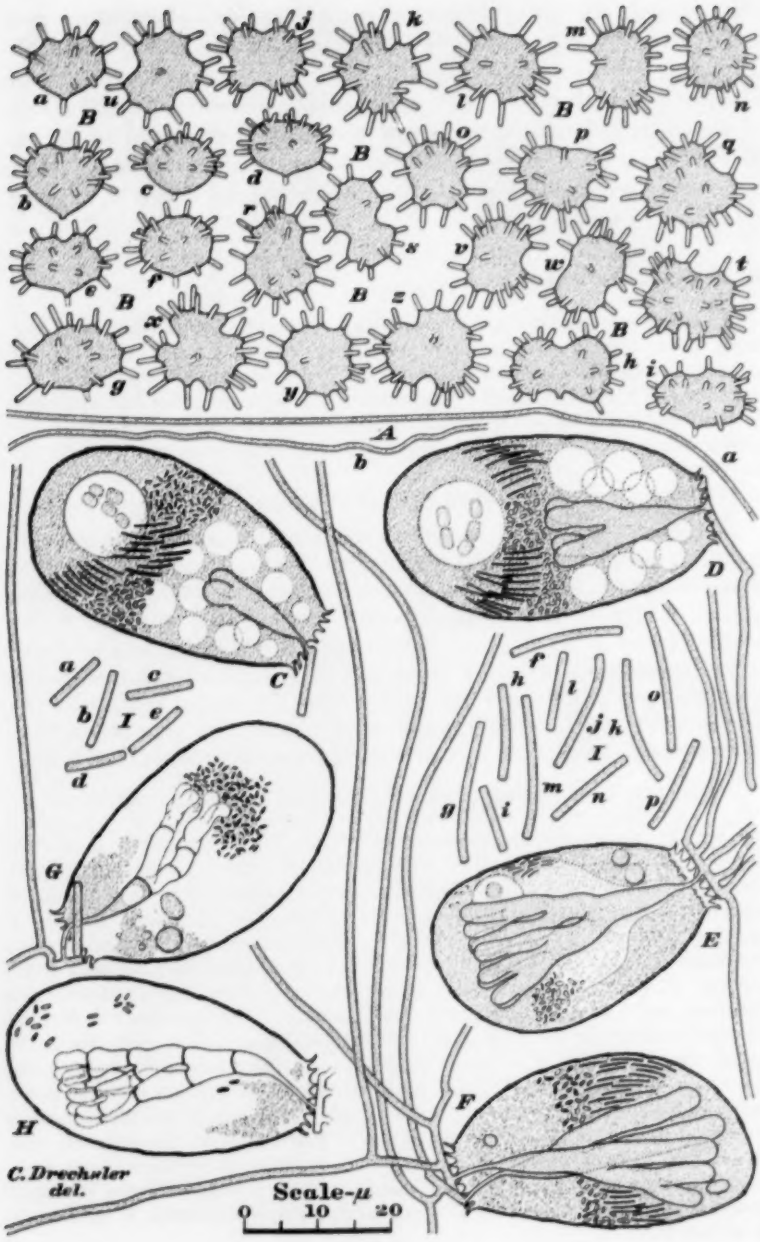

FIG. 2.

Acaulopage bicornis came under observation in several maize-meal agar plate cultures which after being overrun by *Pythium spinosum* had been further planted with small quantities of decaying vegetable detritus collected in southern Louisiana on December 20, 1952. The fungus is at once recognizable as being closely related to my *A. crobylospora* (**10**) owing to strong resemblances in conidial morphology. Some of its smallest conidia bear at the tip a tubular appendage that may remain simple (FIG. 3, *A*, *a*, *b*) or may bifurcate distally (FIG. 3, *B*). Most often in conidia of usual dimensions the tubular appendage dichotomizes proximally (FIG. 3, *C*, *a–z*; *D*) and thus comes to consist of 2 branches generally about one-half as long as the living cell supporting them. The 2 branches commonly diverge at a rather wide angle somewhat like the antennae of certain insects. Now and then one of the 2 branches will bifurcate (FIG. 3, *E*, *a–e*) so that the appendage shows 3 rounded tips, and occasionally both primary branches are found bifurcated (FIG. 3, *F*). In rare instances each of the 2 branches is found bifurcated twice in succession, and one or more terminal elements may even show a third bifurcation (FIG. 3, *G*).

Acaulopage longicornis sp. nov.

Mycelium exiguum sed interdum late extentum; hyphis mycelii incoloratis, continuis, parce ramosis, 0.8–0.9 μ crassis; conidiis sparsim oriundis, incoloratis vel aliquantulum fumidis, elongato-ellipsoideis, apice appendice vacua praeditis, cellula eorum viva plerumque 10–17 μ longa et 5–10 μ crassa; appendice basi ramosa, plerumque ex 2–5 ramis 6–18 μ longis et 0.9–1.6 μ latis constante.

Habitat in materiis plantarum putrescentibus prope College Park, Maryland.

Mycelium scanty though sometimes extensive, composed of aseptate, colorless, meagerly branched, filamentous hyphae measuring 0.8–0.9 μ in width; conidia formed sparsely on surface of substratum, colorless or of

FIG. 2. All parts drawn at a uniform magnification with the aid of a camera lucida; $\times$ 1060 throughout. *A*, Two portions of mycelial hypha, *a* and *b*, of *Acaulopage dactylophora*. *B*, Detached conidia of *Acaulopage dactylophora:* nine, *a–i*, are shown in lateral view; eleven, *j–t*, are shown in top view; six, *u–z*, are shown in bottom view. *C*, Conidium of *Cochlonema explicatum* from whose germ tube a young thallus is growing into a specimen of *Sphenoderia dendata*. *D*, *E*, Specimens of *S. dendata* at successively later stages of invasion by *Cochlonema explicatum*, the invading thallus in each animal coming from a single conidium. *F*, Specimen of *S. dentata* invaded by two thalli of *Cochlonema explicatum*, each coming from a separate conidium; both conidia have put forth several external hyphae. *G*, Depleted shell of *S. dentata* with a nearly evacuated thallus of *Cochlonema explicatum*. *H*, Empty thallus of *Cochlonema explicatum* within a depleted shell of *S. dentata*. *I*, Detached conidia, *a–p*, of *Cochlonema explicatum* showing usual variation in length and width.

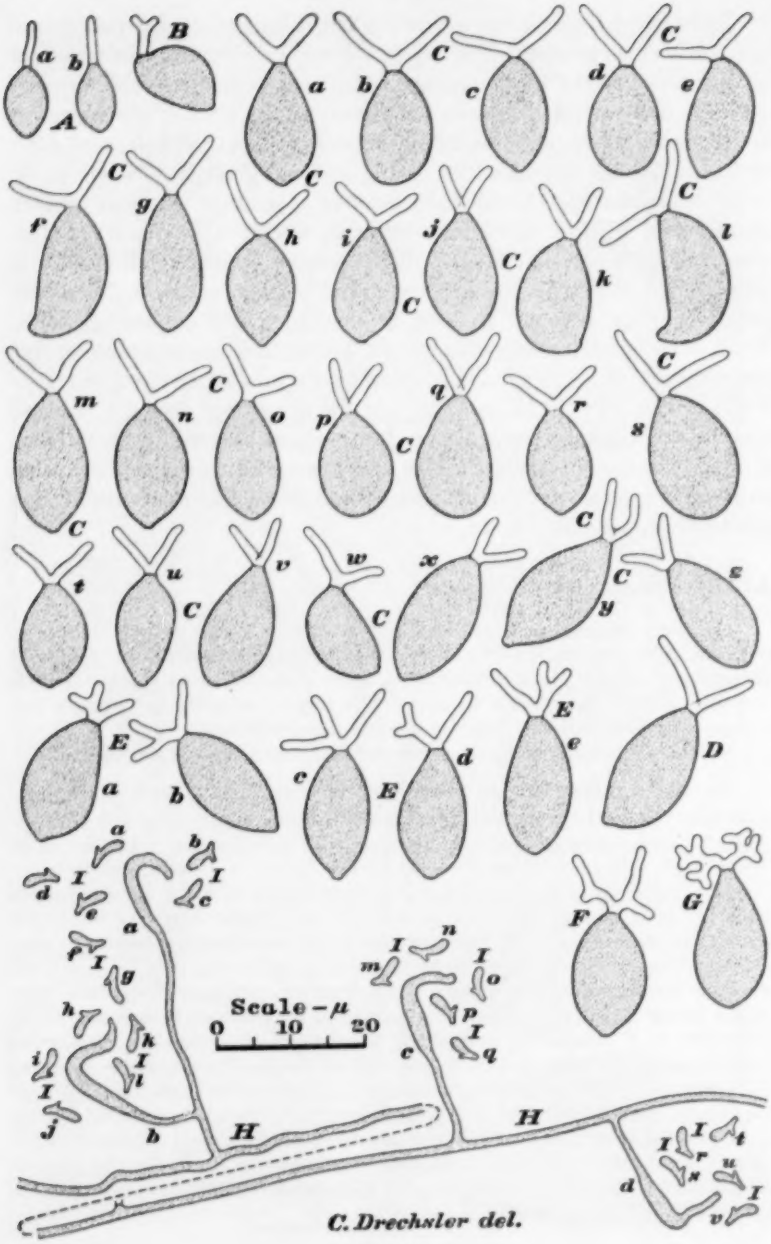

FIG. 3.

a slightly smoky tinge, elongate-ellipsoidal, commonly 10–17 μ long and 5–10 μ wide exclusive of an empty membranous appendage attached at the tip—this appendage proximally ramified and thereby consisting of 2 to 5 (usually of 3) branches 6–18 μ long and 0.9–1.6 μ in greatest width.

Occurring in decaying plant remains near College Park, Maryland.

Acaulopage longicornis developed in some maize-meal agar plate cultures which after being permeated with mycelium of my *Pythium palingenes* had been further planted with leaf mold collected in deciduous woods near College Park early in January, 1951. Its slender hyphae (FIG. 4, *A, B*) are only slightly wider than those of the most delicate species belonging in the Zoopagaceae. The conidia produced on short branches (FIG. 4, *A, a*) or on upcurved terminations (FIG. 4, *B, a*) appear larger than might be expected to originate from such narrow filaments. Usually when a conidium has completed its growth the portion of hypha adjacent to it is soon emptied of protoplasmic contents (FIG. 4, *C, D*) and thereupon gradually becomes indiscernible. Consequently most of the conidia scattered over the surface of a substratum show no visible attachment below.

In *Acaulopage longicornis*, as in the closely related *A. bicornis* and *A. crobylospora*, the conidium bears an empty branched appendage at its tip. Sometimes the appendage consists of only 2 divaricate branches (FIG. 4, *D; E, a–g*) and then resembles the type of appendage most frequent in *A. bicornis*. However, in the present species three-pronged branching (FIG. 4, *F, a–q*) is found considerably more often than bifurcate branching, and seems generally to occur more often also than four-pronged branching (FIG. 4, *G, a–j*). Appendages consisting of 5 branches (FIG. 4, *H, a, b*) are usually observed only in scattered in-

FIG. 3. All parts drawn at a uniform magnification with the aid of a camera lucida; × 1060. *A–G*, Detached conidia of *Acaulopage bicornis* showing variations in size and shape of the living cell as well as in the branching habit of the empty apical appendage: *A*, Small conidia, *a* and *b*, each with an unbranched tubular appendage. *B*, Small conidium with a distally bifurcated appendage. *C* (*a–z*), *D*, Conidia of most usual type, each bearing a proximally bifurcated appendage about one-half as long as the living cell. *E*, Conidia, *a–e*, each bearing an appendage with 3 terminations. *F*, Conidium bearing an appendage with 4 terminations. *G*, Conidium bearing an appendage with 9 terminations. *H*, Reproductive hypha of *Euryancale obliqua* with 4 conidiiferous branches, *a–d*, as seen when their aerial terminations were flattened down under a cover glass; owing to lack of space the hypha is shown in 2 parts whose proper connection is indicated by a broken line. *I*, Detached conidia, *a–v*, of *Euryancale obliqua* shown lying in positions near the branches (*H, a–d*) on which they were formed.

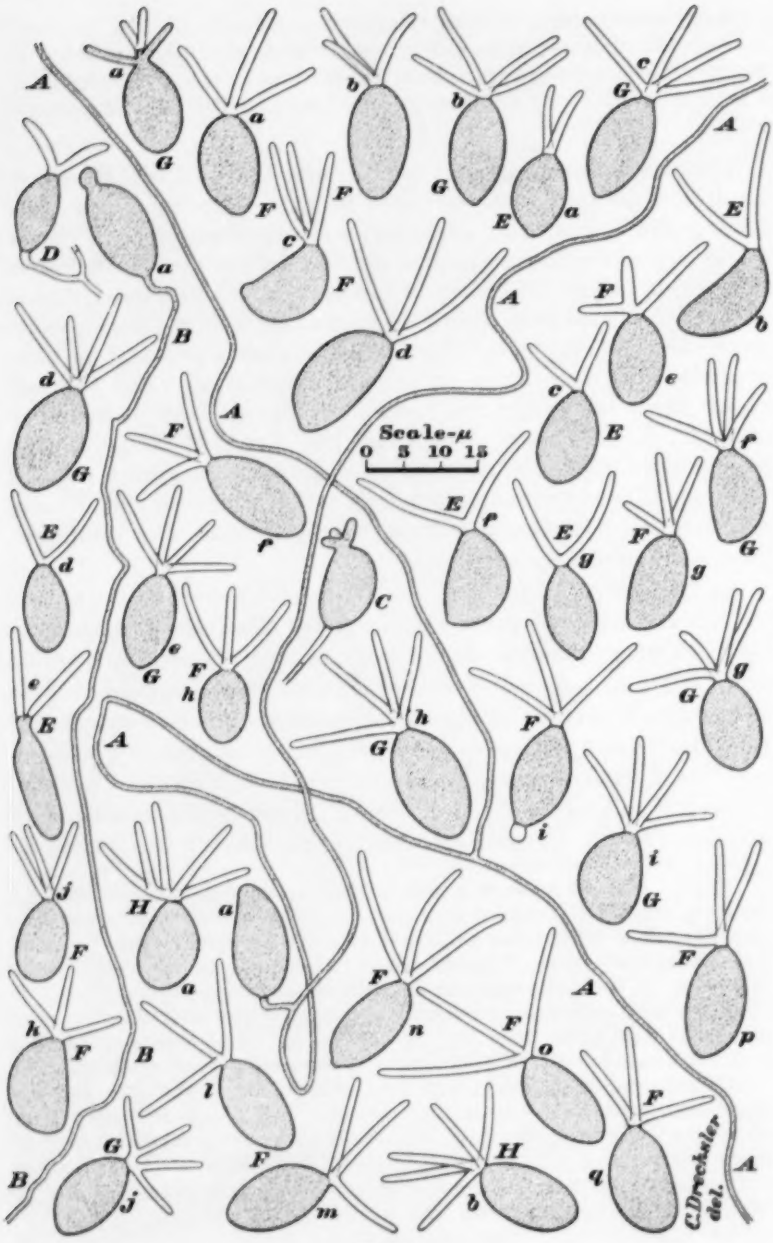

FIG. 4.

stances. Regardless of their variations in number the branches here are approximately equal in length to the living cell supporting them, whereas the corresponding branches in *A. bicornis* are about one-half and in *A. crobylospora* scarcely one-fifth as long as the living cell from which they arise.

Cochlonema explicatum sp. nov.

Ectoparasiticum; hypha assumentis ex tubulo germinationis circa 0.6 μ crasso crescente, usque 3–4.5 μ latescente, semel usque ad quater repetite dichotoma, non convoluta sed animal in longitudinem recte penitus intrante, saepe 27–33 μ longa, ramulis ejus vulgo 2–4 μ latis; conidiis incoloratis, filiformibus, saepius 9–21 μ longis, 1–1.2 μ crassis, in catenulas filiformes ascendentes 150–300 μ longas digestis.

Sphenoderiam dentatam enecans habitat in materiis plantarum putrescentibus prope Willards, Maryland.

Ectoparasitic; assimilative hypha arising from a germ tube about 0.6 μ thick, widening to a diameter of 3–4.5 μ, bifurcating 1 to 4 times, not convolved but penetrating deeply straightforward into the animal, often 27–33 μ long, its branches 2–4 μ wide; conidia colorless, filiform, often 9–21 μ long, 1–1.2 μ wide, borne in ascending chains 150–300 μ long.

Destroying *Sphenoderia dentata*, it occurs in decaying plant detritus near Willards, Maryland.

Cochlonema explicatum came to light as it was destroying about a hundred individuals of *Sphenoderia dentata* Penard (**12**) in a maize-meal agar culture which, after being permeated by mycelium of *Pythium ultimum*, had been further planted with small quantities of decaying vegetable residues collected in eastern Maryland on March 7, 1953. The fungus always began its attack with the germination of one (FIG. 2, *C–E*) or two (FIG. 2, *F*) conidia that had become affixed to the animal host at its aperture. In all observed instances the very slender germ tube grew out from the rounded surface of the spore, sometimes originating near one end (FIG. 2, *C*) and sometimes near the middle

FIG. 4. *Acaulopage longicornis* obtained from material collected near College Park, Maryland; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1060 throughout. *A, B*, Portions of mycelial hypha, each connected with an immature conidium, *a*. *C*, Terminal portion of hypha with a conidium from which a three-pronged appendage is being extended. *D*, Portion of empty mycelium with a mature conidium bearing a proximally bifurcated appendage. *E*, Seven detached conidia, *a–g*, each with a proximally bifurcated appendage. *F*, Seventeen detached conidia, *a–q*, of the type most usual for the species, each being provided with a three-pronged apical appendage about as long as the living cell. *G*, Ten detached conidia, *a–j*, each with a four-pronged appendage. *H*, Two detached conidia, *a* and *b*, each with a five-pronged appendage.

(FIG. 2, *D*, *E*). After penetrating into the host protoplasm a few microns the germ tube would begin widening out to form an assimilative thallus. On attaining a width of 3 to 4.5 μ the thallus would branch dichotomously (FIG. 2, *C*). Further elongation was followed by a second dichotomy (FIG. 2, *D*) and later very often by a third dichotomy (FIG. 2, *E*) of all terminal branches. In host animals of unusually large size a fourth bifurcation in some terminal branches resulted in assimilative thalli with 9 to 12 tips. Apparently absorption of nutrients from the host protoplasm always began at a relatively early stage in the growth of the thallus, for the infective conidium not only remained filled throughout with living contents, but in all instances soon began to put forth hyphae which either directly or after some procumbent or submerged development would grow upward into the air (FIG. 2, *E*, *F*). Production of aerial hyphae destined to be converted into conidial chains continued as the thallus supplied materials obtained by expropriation of the host. Eventually when the host substance was exhausted the thallus yielded up its own contents as it became emptied from distal tips to narrow attachment. Retaining walls laid down at intervals marked successive stages in this progressive evacuation (FIG. 2, *G*, *H*).

Cochlonema explicatum differs conspicuously from all other fungi that have been described as members of the same genus in that its thallus grows straightforwardly without any indication of coiling and without becoming detached from the infective conidium. Moreover, as the infective conidium remains affixed externally to the host animal, where it not only keeps filled with protoplasm but on being supplied with materials taken from the animal soon begins to put forth aerial reproductive hyphae, the fungus would seem to operate as an ectoparasite rather than as an endoparasite. In its relation to the host rhizopod the fungus thus offers some parallelism with my *Bdellospora helicoides* (**1**), but while in the latter species the externally affixed conidium expands into a largish vegetative body the conidium of *C. explicatum* undergoes no marked change in size or shape. After several short branches have grown from the conidium into the substratum to provide anchorage, as it were, for the tall sporogenous filaments, a relationship of parts prevails nearly the same as in species of *Zoopage*. Although the rather massive thallus might conceivably be construed as a haustorium, assignment to *Zoopage* would yet seem inexpedient as the fungus never captures animals by means of its mycelial hyphae but always attacks them after a usual manner of parasites by invading them with germ hyphae from affixed conidia. Since the fungus does not fit well into any established genus it might advantageously be made the

type of a separate genus should one or more closely similar ectoparasitic forms be discovered. Its somewhat provisional assignment to *Cochlonema* was suggested especially by the strong resemblance of its cylindrical or filamentous conidia (FIG. 2, *I*, *a–p*) to the conidia of *C. cylindricum* Drechsl. (**2**), *C. bactrosporum* Drechsl. (**4**), and *C. pumilum* Drechsl. (**5**), which all subsist parasitically on different testaceous rhizopods.

Euryancale obliqua sp. nov.

Mycelium assumens in vermiculo nematoideo crescens, in hyphis continuis, filiformibus, circa 2.5 μ crassis consistens; hyphis genitabilibus ex animali mortuo diverse porrectis, continuis, incoloratis, repentibus, saepe 0.9–1.2 μ latis, ramulos fertiles ad pares angulos proferentibus; ramulis fertilibus saepe 20–35 μ longis, basi repentibus et circa 0.8 μ latis, medio in aere abrupte recurventibus et 2–2.5 μ latis, apice 1.1–1.6 μ latis et ibi conidia deinceps gerentibus; conidiis hyalinis, siliquiformibus, leviter curvatis, 4.5–5.5 μ longis, 1.1–1.3 μ latis, in convexo latere prominentia obliqua 0.5–0.8 μ longa praeditis.

Vermiculos nematoideos necans habitat in materiis plantarum putrescentibus prope Willards, Maryland.

Assimilative mycelium developing in free-living nematodes, consisting of continuous filamentous hyphae approximately 2.5 μ wide; reproductive hyphae extended in different directions from the dead host nematode, mainly prostrate, commonly 0.9–1.2 μ wide, bearing conidiiferous branches at angles approximating a right angle; conidiiferous branches often 20–35 μ in total length, consisting individually of a proximal prostrate filamentous part about 0.8 μ wide, together with a more expanded median part, 2–2.5 μ wide, that curves abruptly upward into the air and terminates in an apical part, 1.1–1.6 μ wide, on which conidia are produced one after another; conidia hyaline, somewhat pod-shaped, 4.5–5.5 μ long, 1.1–1.3 μ wide at the more expanded end, narrowing perceptibly toward the other end, slightly curved, each bearing obliquely on its convex side a protuberance (probably a pedicel) 0.5–0.8 μ long, which usually is directed nearly in alignment with the narrower end.

Parasitic on free-living nematodes, it occurs in decaying plant detritus near Willards, Maryland.

Euryancale obliqua came under observation in a maize-meal agar plate culture which after being overgrown by *Pythium ultimum* had been further planted with a small quantity of vegetable detritus collected in eastern Maryland on March 7, 1953. Development of the fungus was limited to the bodies of a few eelworms, none in a condition suitable for identification. For the most part the divaricate hyphae and their

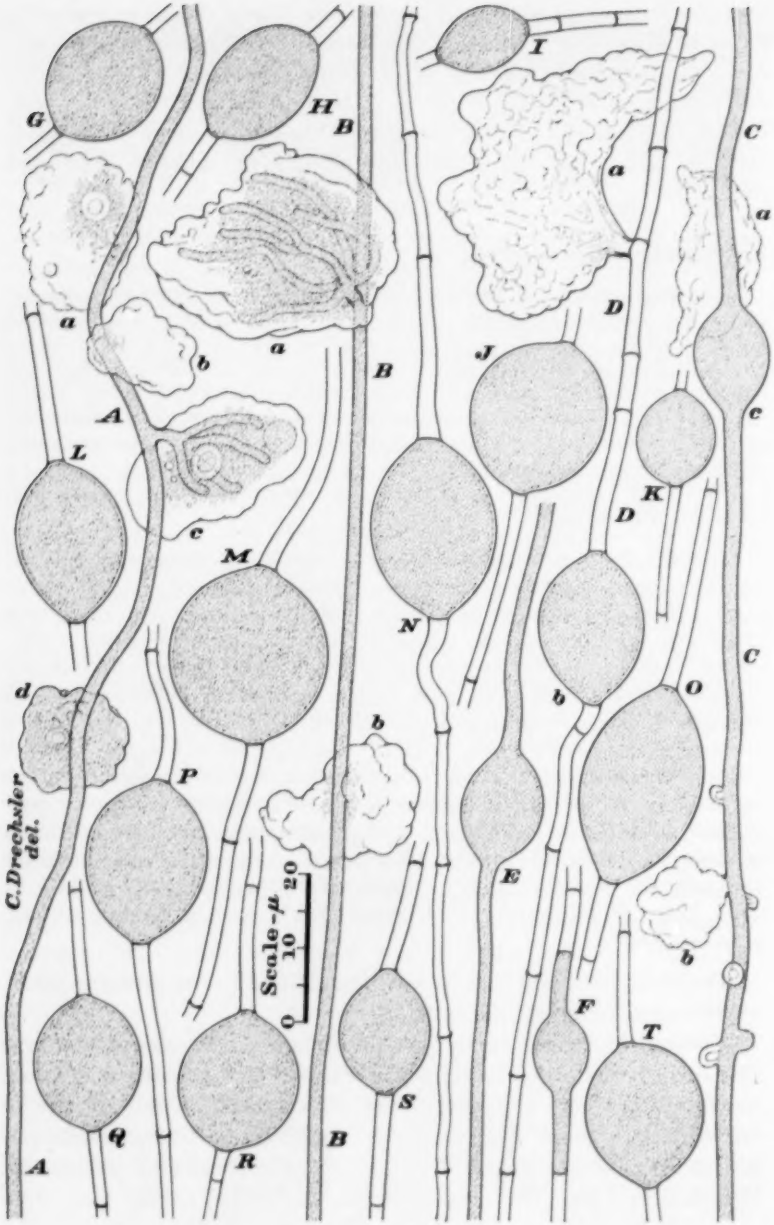
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FIG. 5.

conidiiferous branches (FIG. 3, *H*, *a–d*) were very badly mangled from the bustling of numerous eelworms present in the culture, though enough remained in a tolerable state to permit ready recognition of a general parallelism with the asexual reproductive habit of my *E. sacciospora* (**5**). The conidia (FIG. 3, *I*, *a–v*) strewn abundantly on the substratum bore no empty appendage, and thereby differed markedly from the conidia of *E. sacciospora* as well as from those of the unnamed congeneric species earlier observed in a culture prepared with leaf mold from Wisconsin (**11**: 184–185). In their unsymmetrical pod-like shape, modified by the minute spur on the convex side, as also in their small dimensions, they resembled curiously the conidia of my *Harposporium diceraeum* (**7**: 797–799), a nematode parasite of widely different taxonomic relationship.

Cystopage ellipsospora sp. nov.

Mycelium exiguum sed saepe late extentum; hyphis ejus continuis, incoloratis, parce ramosis, plerumque 1.7–2.5 μ latis, ad amoebas inhaerentibus, animalia ita capientibus, pelliculam cujusque captivi perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio ad instar arbusculae penitus extenso, in ramulis 1–1.5 μ crassis consistente; chlamydosporis incoloratis vel leviter flavidulis vel leviter fumidis, vulgo intercalaribus, globosis vel elongato-ellipsoideis, plerumque 12–28 μ longis, 8–23 μ latis.

Amoebas saepe 15–50 μ latas capiens consumensque habitat in materiis plantarum putrescentibus prope Laplace, Louisiana.

Mycelium scanty but often widely extended; mycelial hyphae aseptate, colorless, rather meagerly branched, mostly 1.7–2.5 μ wide, capturing amoebae through adhesion, penetrating the pellicle of each captive and intruding deeply a bush-like haustorium consisting of assimilative branches mostly 1–1.5 μ wide; chlamydospores colorless or faintly yellowish or faintly smoky, commonly formed intercalarily in axial alignment with the parent hypha, sub-globose or often elongated-ellipsoidal, mostly 12–28 μ long and 8–23 μ wide.

FIG. 5. *Cystopage ellipsospora*, drawn at a uniform magnification with the aid of a camera lucida; ×1060 throughout. *A*, Portion of hypha with 4 captured amoebae, *a–d*; two amoebae, *a* and *b*, have been emptied of nearly all their contents; one, *c*, is extensively invaded by a haustorium; and the fourth, *d*, is undergoing invasion by a haustorium. *B*, Portion of hypha with 2 captured amoebae, *a* and *b*; one amoeba, *a*, being extensively invaded by a bush-like haustorium; the other, *b*, present only as an empty pellicle. *C*, Portion of hypha showing the empty pellicles of 2 captured amoebae, *a* and *b*, and also a young chlamydospore, *c*. *D*, Portion of hypha with the collapsed pellicle of a captured amoeba, *a*, and a delimited chlamydospore, *b*. *E*, Portion of hypha with a young enlarging chlamydospore. *F*, Portion of hypha in which formation of a small chlamydospore is nearing completion. *G–T*, Chlamydospores in definitive state, showing usual variation in size, shape and axial alignment.

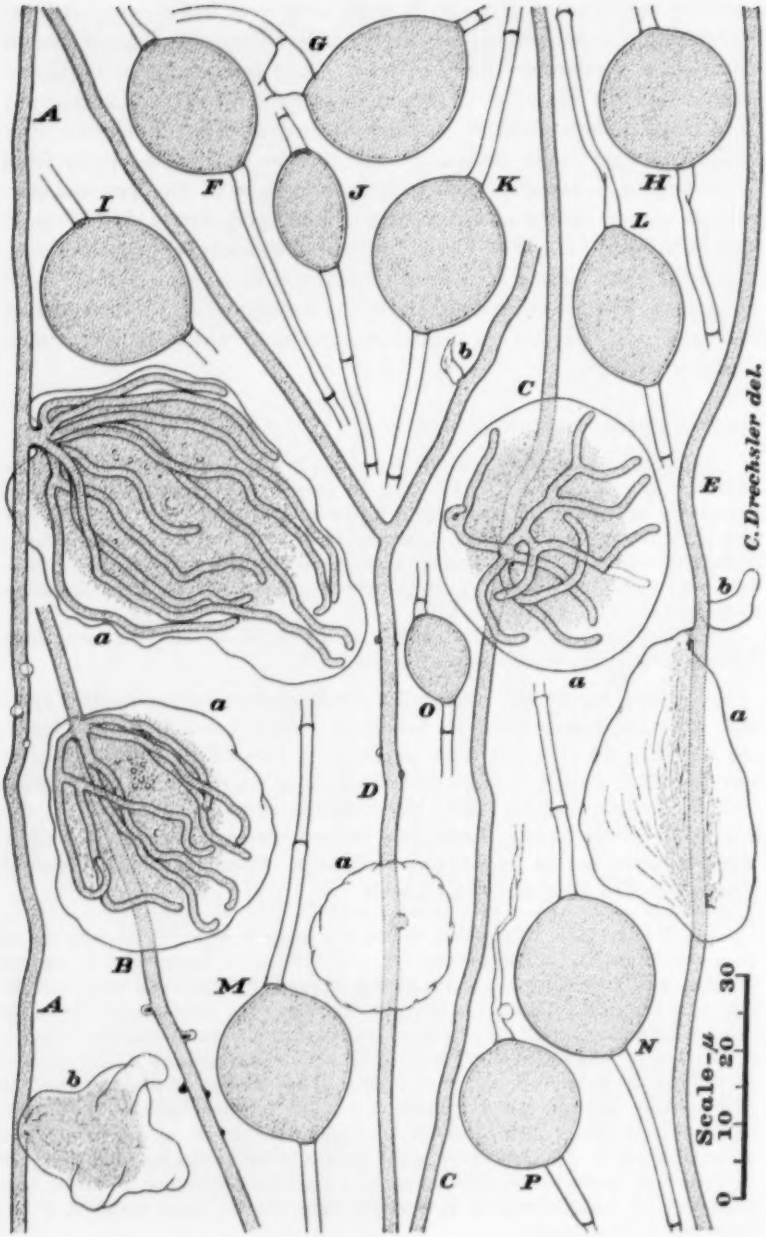
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FIG. 6.

Capturing and consuming amoebae commonly 15 to 50 μ wide, it occurs in decaying plant materials near Laplace, Louisiana.

Cystopage ellipsospora came under observation in more than a dozen maize-meal agar plate cultures to which had been added small quantities of decaying plant detritus gathered in southern Louisiana on December 20, 1952. In these cultures its sparse mycelium extended over large areas. The individual filaments (FIG. 5, *A–C;* FIG. 6, *A–E*) seem little given to abrupt changes in direction or to pronounced fluctuations in thickness. They are appreciably coarser than the hyphae of my *C. subtilis* (**6**: 253–256) but only about one-half as wide as those of the 2 nematode-capturing species I described under the binomials *C. lateralis* (**6**: 248–253) and *C. intercalaris* (**8**: 1–7). To capture amoebae (FIG. 5, *A, d*) the fungus employs an adhesive secretion that often becomes visible on the surface of hyphae as small yellowish lumps. In the area where an animal is held fast the hypha puts forth a lateral process that penetrates the captive's pellicle and then ramifies through the interior to form a bush-like haustorium (FIG. 5, *A, c; B, a.* FIG. 6, *A, a, b; B, a; C, a*). The animal's protoplasm now gradually diminishes (FIG. 5, *A, a*) until the membranous pellicle is very largely if not entirely empty (FIG. 5, *A, b; B, b; C, a, b; D, a.* FIG. 6, *D, a, b; E, a, b*). Ultimately the contents of the assimilative branches are withdrawn backward into the parent hypha, whereupon the empty haustorium together with the collapsed pellicle vanishes from sight. Stumpy protuberances on the hyphae (FIG. 5, *C;* FIG. 6, *A, B, D*) sometimes mark the places where amoebae were captured and destroyed.

Globose swellings (FIG. 5, *C, c; E*) eventually appear here and there in the older mycelial filaments. These swellings gradually enlarge as they receive materials from the adjacent portions of hypha (FIG. 5, *F*). Retaining walls marking successive stages of evacuation are laid down at intervals in both the proximal and distal hyphal portions (FIG. 5, *D*). When movement of protoplasm has been completed the swellings form definitive boundary walls at both ends. Most of the chlamydospores (FIG. 5, *G–T;* FIG. 6, *F–P*) thus brought into being are noticeably greater in length than in width, so that an ellipsoidal rather than a

FIG. 6. *Cystopage ellipsospora*, drawn at a uniform magnification with the aid of a camera lucida; × 1060 throughout. *A*, Portion of hypha with 2 captured amoebae, *a* and *b*, each extensively invaded by a haustorium. *B, C*, Portions of hypha, each with a captured amoeba, *a*, extensively invaded by a haustorium. *D, E*, Portions of hypha to each of which are affixed the empty pellicles of 2 captured amoebae, *a* and *b*. *F–P*, Chlamydospores in definitive state showing usual variations with respect to size, shape, and axial alignment.

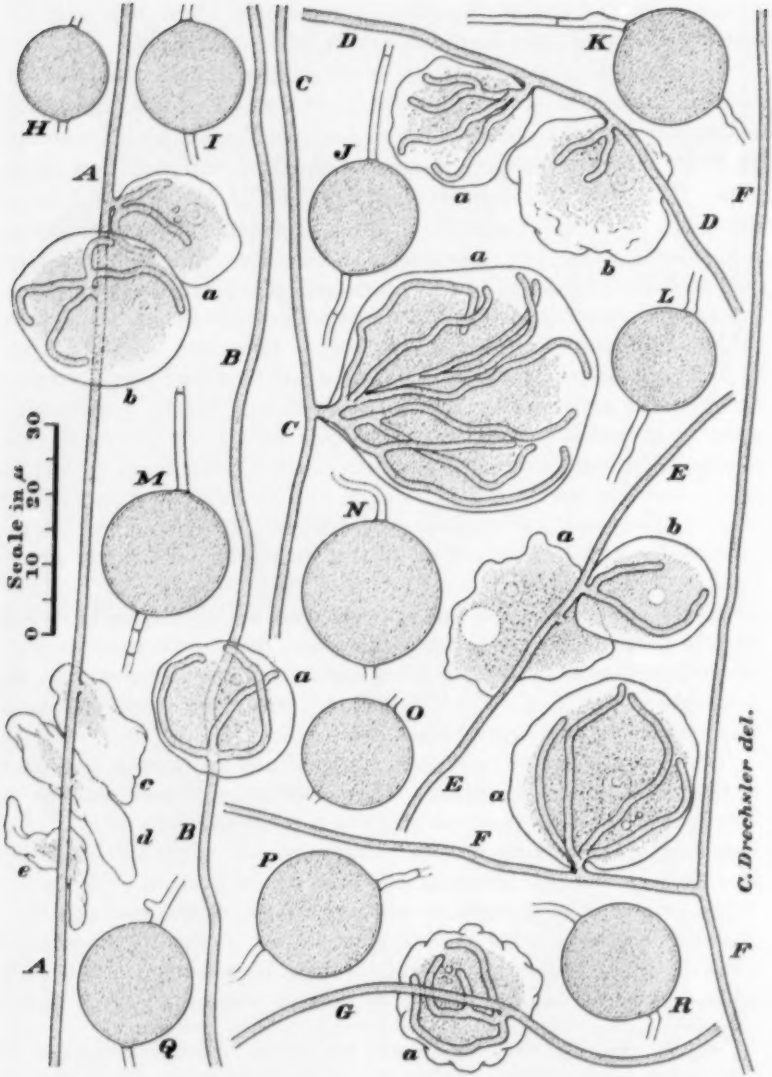

FIG. 7. *Cystopage sphaerospora* obtained from leaf mold collected near Annapolis, Maryland; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Portion of mycelial hypha with 5 captured amoebae, *a–e*; 2 captives, *a* and *b*, show invasion by haustoria, while the 3 others, *c–e*, are present only as depleted pellicles. *B*, *C*, Portions of hypha, each showing one

spherical shape may be considered typical of these meagerly expressive reproductive bodies. It is also noteworthy that the chlamydospores of *Cystopage ellipsospora*, like those of *C. intercalaris*, usually appear in axial alignment with the parent hyphae, though in scattered instances they are borne somewhat laterally (FIG. 6, *G*, *I*). They are appreciably smaller than the chlamydospores of *C. lateralis* and *C. intercalaris*, but larger than those of *C. subtilis*. In the fine texture of their contents as well as in the faintly yellowish or smoky tinge often noticeable in them they resemble not only the chlamydospores of congeneric forms but also the more massive of the conidia produced in other genera of the Zoopagaceae.

Cystopage sphaerospora sp. nov.

Mycelium exiguum sed saepius late pansum; hyphis ejus continuis, incoloratis, parce ramosis, plerumque 1–1.8 μ crassis, ad amoebas inhaerentibus, animalia ita capientibus, pelliculam cujusque captivi perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio ad instar arbusculae penitus extenso, ex ramulis 1–1.2 μ constante; chlamydosporis incoloratis vel leviter flavidulis vel leviter fumidis, vulgo intercalaribus, plerumque sphaericis, 9–21 μ crassis.

Amoebas saepe 15–35 μ latas capiens consumensque habitat in humo silvestri prope Annapolis, Maryland, et prope Moorestown, New Jersey.

Mycelium scanty but often spreading extensively; mycelial hyphae aseptate, colorless, sparingly branched, mostly 1–1.8 μ wide, capturing amoebae by adhesion, penetrating the pellicle of each captive and intruding deeply a bush-like haustorium consisting of assimilative branches mostly 1–1.2 μ wide; chlamydospores colorless or faintly yellowish or faintly smoky, commonly formed intercalarily in axial alignment with the parent hypha, mostly spherical, 9–21 μ in diameter.

Capturing and consuming amoebae commonly 15 to 35 μ wide, it occurs in leaf mold in deciduous woods near Annapolis, Maryland, and near Moorestown, New Jersey.

Cystopage sphaerospora was found developing in several maize-meal agar plate cultures which after being overgrown by mycelium of *Pythium spinosum* had been further planted with small quantities of leaf mold taken from deciduous woods in central Maryland on February 27, 1954. It likewise developed in some maize-meal agar plate cultures of *P. spino-*

captured amoeba, *a*, extensively invaded by a haustorium. *D*, Portion of hypha with 2 captured amoebae, *a* and *b*, each invaded by a haustorium. *E*, Portion of hypha with a newly captured living amoeba, *a*, and another amoeba, *b*, invaded by a haustorium. *F*, *G*, Portions of hyphae, each with a captured amoeba, *a*, extensively invaded by a haustorium. *H–R*, Chlamydospores in definitive state, showing usual variations in size, shape, and axial alignment.

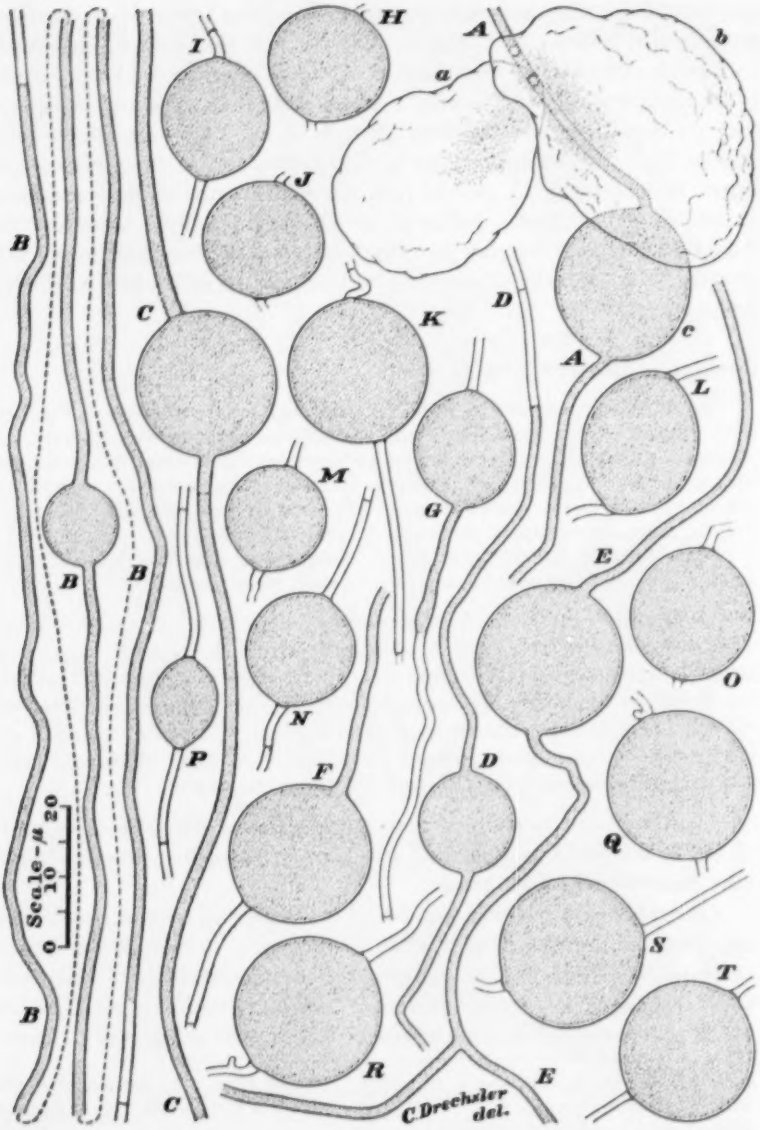

FIG. 8. *Cystopage sphaerospora* obtained from leaf mold collected near Annapolis, Maryland; all parts drawn at a uniform magnification with the aid of a camera lucida; ×1000 throughout. *A*, Portion of mycelial hypha with depleted pellicles of 2 captured amoebae, *a* and *b*, as well as with a growing chlamydospore, *c*. *B*,

sum to which had been superadded small quantities of leaf mold collected in deciduous woods in western New Jersey on February 25, 1954. The fungus closely resembles *C. ellipsospora* in its manner of capturing pelliculate amoebae (FIG. 7, *E, a*), in the bush-like branching of the haustoria it intrudes into the captives (FIG. 7, *A, a, b; B, a; C, a; D, a, b; E, b; F, a; G, a*), and in the withdrawal of protoplasm from each haustorium after the animal's contents have been expropriated (FIG. 7, *A, c–e;* FIG. 8, *A, a, b*). It is, however, a somewhat less robust species as its mycelial hyphae (FIG. 7, *A–G;* FIG. 8, *A–G*) are appreciably narrower than those of *C. ellipsospora* and therefore conspicuously narrower than the hyphae of *C. lateralis* and *C. intercalaris*. While in *C. sphaerospora* the haustorical branches are generally a little slenderer than the mycelial filaments from which they originated, the difference in width here is less pronounced than the corresponding dimensional difference in *C. ellipsospora*.

The chlamydospores of *Cystopage sphaerospora* are formed, much like those of *C. ellipsospora* and other congeners, through withdrawal of protoplasm from extensive portions of hypha into intercalary swellings (FIG. 8, *A, c; B–G*). When they are fully developed (FIG. 7, *H–R;* FIG. 8, *H–T*) their preponderantly spherical shape provides a distinguishing feature of the species, though some individuals are usually to be found in which either the length noticeably exceeds the width (FIG. 8, *I, L, P*) or the width noticeably exceeds the length (FIG. 7, *M;* FIG. 8, *H, J*). They are decidedly smaller than the chlamydospores of *C. intercalaris* or of *C. lateralis*, somewhat smaller than those of *C. ellipsospora*, and somewhat larger apparently than those of *C. subtilis*. As a rule they are formed intercalarily in axial alignment with the parent hypha and thus differ widely with respect to positional relationship from the frequently lateral and laterally intercalary chlamydospores of *C. subtilis*.

A COARSE AMOEBA-CAPTURING ZOOPAGACEOUS FUNGUS THAT PRODUCES ZYGOSPORES

Occasion was taken earlier (8: 7–9) to discuss a sexual reproductive stage which it was held might possibly belong to *Cystopage intercalaris*

Long hyphal segment the contents of which are migrating into the chlamydospore developing near the middle. *C–E*, Portions of hyphae, each with a growing chlamydospore that is still receiving protoplasmic materials at both poles. *F, G*, Two chlamydospores, each delimited at one pole, but still receiving materials at the other pole. *H–T*, Chlamydospores in definitive state, showing usual variations in size, shape, and axial alignment.

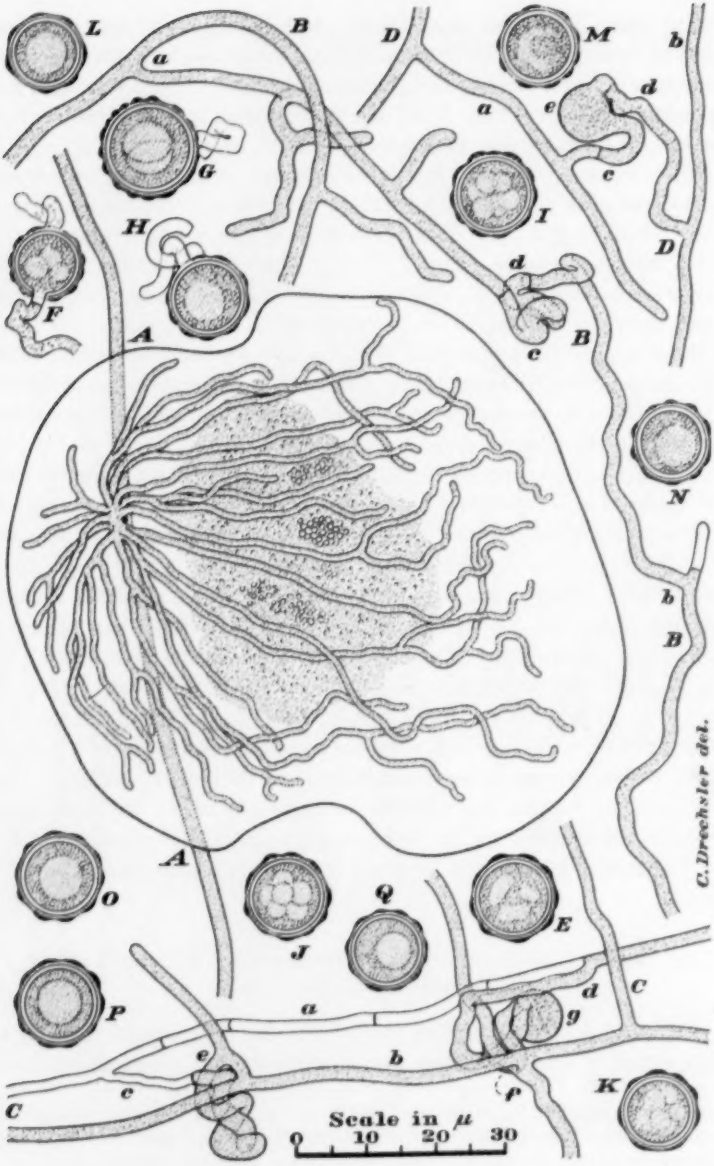
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FIG. 9.

since it was produced by a similarly coarse zoopagaceous fungus and since, further, it yielded crustose zygospores differing a little from the zygospores known in any related conidial genus. During subsequent years no sexual stage has been definitely established for any species of *Cystopage*, and no asexual reproductive stage has been found connected with crustose zygospores. As neither *C. ellipsospora* nor *C. sphaerospora* is known to have a sexual stage it may be opportune to record the production of crustose zygospores by an amoeba-capturing zoopagaceous form that appeared in a maize-meal agar plate culture which after being overgrown with *Pythium* mycelium had been further planted with leaf mold collected near Haugen, Wisconsin, in September, 1939.

The scanty yet rather extensive mycelium of the predacious fungus here in question was composed of colorless, aseptate, sparingly branched hyphae ranging in width from 1.5 to 2.5 μ (FIG. 9, *A*). To these hyphae were affixed here and there specimens of a pelliculate *Amoeba* measuring 50 to 100 μ across. In some of the captured animals the degenerating nucleus could still be recognized as having the dimensions and internal organization characteristic of the nucleus in *A. verrucosa* Ehrenberg. Expropriation of the animal's contents was accomplished by means of an extensively ramified bush-like haustorium (FIG. 9, *A*) rather closely resembling the haustorium of my *Zoopage mitospora* (**3**). The main branches making up the haustorium sometimes exceeded 100 μ in length. Usually about 1.5 μ wide near the base, they narrowed very gradually to a distal width of 0.8 or 0.9 μ in their several delicate terminations.

Zygospore development in the fungus came from conjugation of 2 sexual branches (FIG. 9, *B, c, d; C, c, e; C, d, f; D, c, d*) given off from different main hyphae (FIG. 9, *B–D: a, b*) having no close mycelial connection with one another. Some pairs of branches were found

FIG. 9. Zoopagaceous fungus producing zygospores of crustose type held possibly referable to *Cystopage;* all parts drawn at a uniform magnification with the aid of a camera lucida; ×1000 throughout. *A*, Portion of mycelial hypha from which a richly branched bush-like haustorium has extensively invaded a large pelliculate captive amoeba. *B*, Two main hyphae, *a* and *b*, that have given off, respectively, the 2 sexual branches *c* and *d*, which are shown in apical contact. *C*, Two young reproductive units originating from the same 2 main hyphae, *a* and *b:* the sexual branches *c* and *d* given off by hypha *a* have paired, respectively, with the branches *e* and *f* given off by hypha *b;* a globose body, *g*, is developing from the union of *d* and *f*. *D*, Two main hyphae, *a* and *b*, that have given off, respectively, the sexual branches *c* and *d* which have united to produce the globose body, or young zygosporangium, *e*. *E–Q*, Crustose zygospores showing usual variations in size, shape and internal organization.

spirally intertwined (FIG. 9, *C, c, e*) and others showed a more haphazard type of engagement (FIG. 9, *B, c, d*). A terminal cell or gametangium most often 12 to 20 μ in length was delimited in each branch at about the time or perhaps shortly before the 2 branches became fused at their tips. Soon afterwards a globose distention (FIG. 9, *C, g; D, e*) began developing in the fusion cell and usually near the place of union. In view of the evident dimensional relationships there is good reason to presume that apart from the contents of the fusion cell a considerable quantity of material from one or both sexual branches was received into the globose distention before it attained definitive size and began to be converted into a zygospore. The globose reproductive bodies (FIG. 9, *E–Q*), mostly varying from 10.5 to 15 μ in diameter, eventually acquired a wall composed of 2 layers, the inner layer appearing colorless and smoothly spherical, while the outer one was yellow and presented an irregularly undulating peripheral outline. Zygospores in a resting condition usually showed a single reserve globule (FIG. 9, *H, L, N–Q*) often 5 to 6.5 μ wide, or several smaller globules, within a parietal layer of densely granular protoplasm.

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STUDIES IN THE GENUS SPORONEMA

DONALD P. LIMBER [1]

(WITH 3 FIGURES)

The writer's interest in the genus *Sporonema* followed the repeated interception of a fungus described as *Excipula diapensiae* Rostr. In studying the possibility of placing this species in *Sporonema*, the heterogeneous nature of the species heretofore assigned to the genus became apparent. The writer believes that the following account will be of interest to others. Heterogeneity in the genus has been noted before. It is in part due to von Hoehnel's (**5**) criticism of certain species that Diedicke (**3**, p. 743) included only *S. phacidioides* and *S. catalpae* in *Sporonema*. Descriptions of at least twenty-nine species appear in the literature. A number of them have been transferred to other genera, but nineteen species remain in the form genus *Sporonema*.

Species of *Sporonema*, like other *Discellaceae*, are distinguished by pycnidia which open by a split or lacination of the wall, and a cupulate or disc form at maturity. These characters are often poorly developed and some species are not easily distinguished from certain species in genera of other families. The shape of the pycnidia may be nearly spherical at first, but it is usually depressed or lenticular at maturity before opening. After opening the cupulate or disc-like character is evident.

The notes that follow are the result of the study of herbarium specimens with the exception of those on *S. oxycocci* and *Excipula diapensiae*, for which fresh collections were available. *S. oxycocci* was grown in culture. The species are discussed in alphabetical order with the exceptions of *S. phacidioides*, the type, and certain species that have been transferred to other genera previously.

[1] The writer is indebted to John A. Stevenson, curator of the National Fungus Collections, of the Horticultural Crops Research Branch, Agricultural Research Service, for the loan of specimens and criticisms of the manuscript; to D. P. Rogers and the New York Botanic Garden for the use of specimens and bibliographical assistance; to I. McKenzie Lamb, curator of the Farlow Reference Library and Herbarium of Cryptogamic Botany, for the loan of specimens; and to Miss E. K. Cash, of the Horticultural Crops Research Branch, for editing the Latin diagnosis and other assistance. Responsibility for the observations and opinions is the author's.

Desmazières (**1**) described the genus *Sporonema* in 1847. A translation of the description as emended by him (**2**) in 1851 follows:

Pycnidia innate, covered by the epidermis, then erumpent, sessile, subimmersed, at first closed then dehiscent from the center to the margin in many pieces. Nucleus discoid, gelatinous, base "diophorus." Conidiophores filiform, simple or dichotomous, one to many-spored. Spores hyaline, straight, ovoid or cylindric. Fungi minute on stems and leaves.

S. phacidioides Desm. was the first species published. It may therefore be accepted as the type of the genus. Jones (**6**) demonstrated that the perfect state of this species is *Pyrenopeziza medicaginis* Fckl.

SPORONEMA PHACIDIOIDES Desm.

This fungus causes yellow-leafblotch of alfalfa. In addition to the specimens in the herbarium of the Plant Quarantine Branch of the Agricultural Research Service, at Hoboken, N. J., two collections in the New York Botanical Garden herbarium were studied, C. Roumeguère Fungi selecti exsiccati *5993* and a specimen collected at Ashland, Oreg., August 28, 1913, by H. S. Jackson. These collections agree with Desmazières' description of the species. In the first description of the genus, describing the *Sporonema,* Desmazières calls the pycnidium membranous, but this is not repeated in the emended description nor in his description of *S. phacidioides.* Von Hoehnel (**5**) called the pycnidia pseudopycnidial and mentions that the wall is incomplete. Jones (**6**) stated that "next to the epidermis there may be a few large somewhat circular, dark-colored cells which do not form a complete protective layer." In the collections examined a complete upper wall could be found in some of the larger pycnidia (FIG. 1, *A*). In such pycnidia the upper wall is nearly black and is composed of large thick-walled cells, some of which are polygonal and others are elongated and interwoven. An incomplete or rudimentary upper wall is more typical of the species. At the sides and base the wall is thin, two to four cells thick, and is concolorous with the host tissue.

SPORONEMA CAMELLIAE Earle.

Three specimens of *S. camelliae* from the N. Y. Botanical Garden herbarium and one specimen from the National Fungus Collections were examined. Each of these specimens is marked "type," although they are not all from the same collection. All were collected in March and April, 1896, on *Camellia japonica,* at Auburn, Lee County, Ala. The figures and data reported below were taken from the specimen labeled: *"Sporo-*

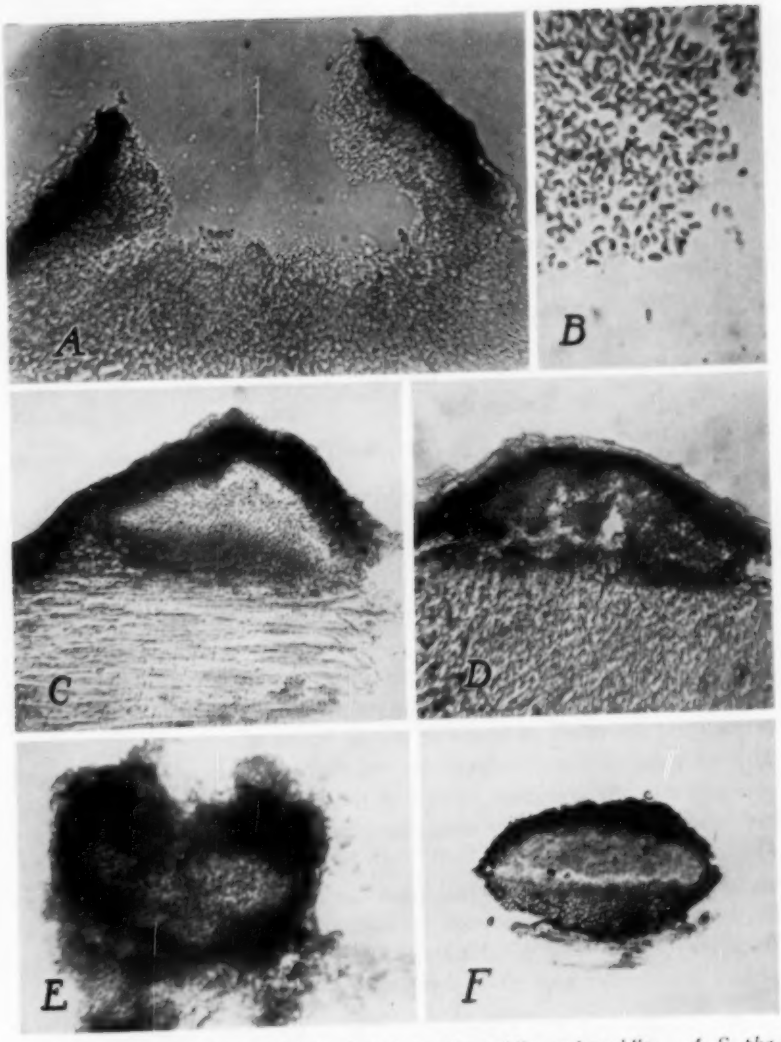

FIG. 1. *Sporonema* spp. Vertical sections of pycnidia, and conidia. *A. S. phacidioides*, × 275. *B. S. laricinum conidia*, × 950. *C. S. fraxini*, × 280. *D. S. glandicola*, × 280. *E. S. platani*, × 260. *F. S. laricinum*, × 100.

nema camelliae Earle, *Camellia japonica,* Auburn, Lee County, Ala., 4/16/1896, F. S. Earle and L. M. Underwood."

The pycnidia are dark and depressed-globose at first, but eventually become lenticular and open widely. They are seated on the palisade cells. The wall is thick at the base and in young pycnidia there may be dark, thick wall tissue above and at the sides (FIG. 3, *S*). In large, mature pycnidia little or no trace of the upper wall can be found, even when the epidermis still partly covers the disc. At this stage the fungus could easily be mistaken for a *Gloeosporium.* The conidia are borne on a palisade of short conidiophores arising from the base. They measured 12–16 × 4–5 μ. They are cylindric or slightly clavate with rounded or, occasionally, almost acute ends (FIG. 2, *L*).

There are two collections of *Sporonema* on *Camellia japonica* in the herbarium at Hoboken, N. J., that have narrower conidia but in other respects agree with *S. oxycocci* Shear. One of them is from England, the other from France.

Sporonema campanulae (DC.) Petr.

Synonyms listed by Petrak (**8**) indicate that *S. campanulae* was first described by DeCandolle as *Xyloma campanulae,* transferred by Fries to *Dothidea,* considered by Fuckel to be a *Phyllachora,* and finally placed in *Placosphaeria* by Bäumler.

The specimen in Petrak's Flora Bohemiae et Moraviae Exsiccata (II Ser., 1. Abt., Pilze Nr. *48*) was examined. The large pycnidia are very clearly the result of fusion or partial fusion of many small pycnidia. This may be indicated merely by the crenate margin of the merged pycnidia and the irregular nature of the cavity, or in less completely fused pycnidia the different component pycnidia may be seen distinctly (FIG. 3, *P*). The adjacent walls may be complete and separate, fused, or in part missing. Individual pycnidia are sphaeropsidaceous. The black covering of the group of pycnidia or locules suggests a clypeus. It is very thin. The conidia measure 2.5–5 × 0.6–1 μ. The conidiophores are slender and 10–15 μ in length.

If Bäumler's assignment of the fungus to *Placosphaeria* is accepted one must note that the conidiophores are rather long. Shortness or the lack of conidiophores is the basis for separation of *Placosphaeria* from *Dothiorella* in Clements and Shear's key. *Placosphaeria* seems the more suitable, as *Dothiorella* almost invariably develops on stems or thick leaves.

EXCIPULA DIAPENSIAE Rostr.

Rostrup (**9**) described this fungus from the leaves of *Diapensia lapponica* L. It has been collected at the Plant Quarantine Branch inspection house at Hoboken, several times on the leaves of *Shortia*, from Japan, and on *Schizocodon ilicifolius* Maxim. from England. Both hosts are in the family Diapensiaceae. The pycnidia are very small, numerous, and oval in elevation, sometimes appearing conic when the wall is thrust upward after opening (FIG. 3, *Q*). The wall is black and rather uniform in thickness or sometimes thinner at the top. The individual cells cannot be distinguished in the mature pycnidium wall. The conidia and conidiophores are as in *S. oxycocci* but smaller. Since the generic name *Excipula* was first applied to an ascomycete, it cannot be used again for this imperfect fungus. It is proposed that *E. diapensiae* Rostr. be transferred to *Sporonema* with an emended description.

Sporonema diapensiae (Rostr.) comb. nov.

Excipula diapensiae Rostr. Öst. Grönland. Svamp. Med. Grönl. **18**: 71. 1896.

Pycnidia gregarious, minute, amphigenous, 47–75 μ in diam. and 24–36 μ in height, black, lenticular to hemispheric, opening by one or more splits or tears; conidiophores short, simple, about the width of the conidia; conidia hyaline, continuous, cylindric with obtuse ends, usually straight, 10–15.5 × 2.5–3 μ.

Hab. in Diapensiaceae, causing spots in the living leaves, in Denmark, Japan, and England.

Sporonema fraxini sp. nov.

Pycnidiis erumpentibus, hypophyllis, atris, conicis vel hemisphaericis, initio clausis dein a centro irregulariter dehiscentibus et diffractis, parietibus crassis, contextu pseudoparenchymatico, superne atris, inferne dilute olivaceis, nervicolis; conidiophoris obclavatis vel subulatis, 5–8 μ longitis, 1.5–2 μ diam. apud basim, verticem versus attenuatis; conidiis oblongis utrimque obtusis, continuis, hyalinis, 4.5–7 × 1.6–2 μ.

Hab. in foliis *Fraxini oreganae*.

The pycnidia are epiphyllous on tan areas and are almost always seated on a vein. They are black, round or oval, numerous, opening widely to expose the tan disc; upper wall black, thick; lower wall olivaceous; 84–224 μ in diameter; conidiophores obclavate or subulate 5–8 μ long by 1.5–2 μ thick at the base, narrower toward the apex; conidia hyaline, oblong with obtuse ends, continuous, 4.5–7 × 1.6–2 μ.

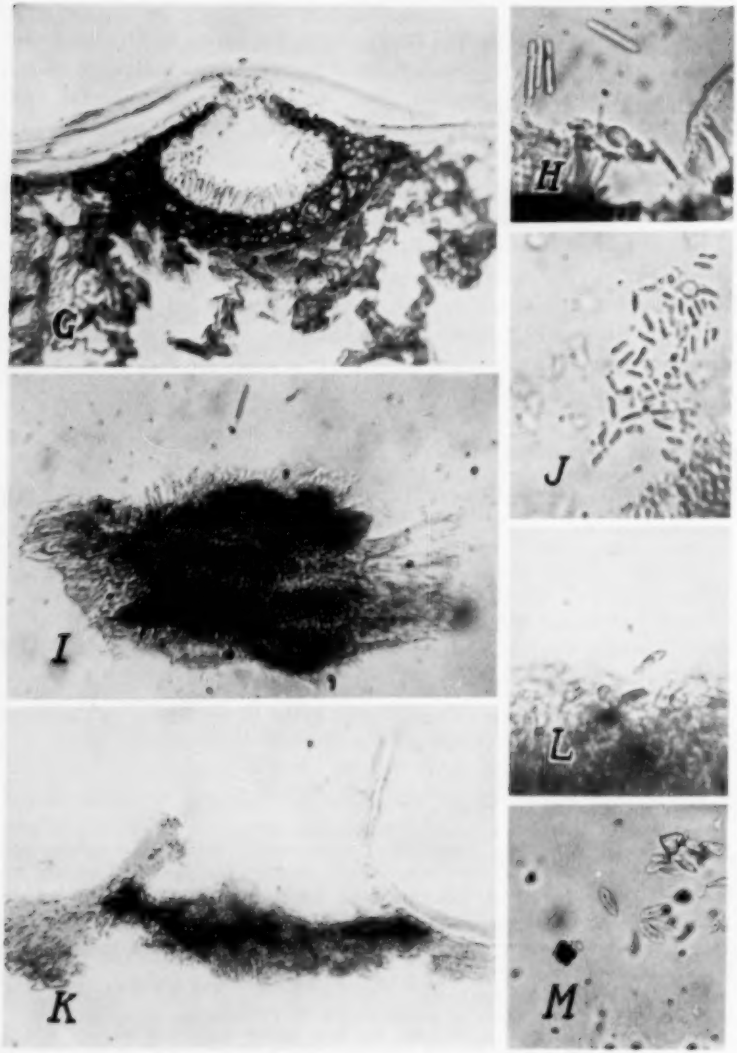

FIG. 2. *Sporonema* spp. Pycnidia (sectioned), and conidia. *G. S. oxycocci*, × 280. *H. S. oxycocci* conidia, × 570. *I. S. rameale*, × 260. *J. S. punctiforme* conidia, × 950. *K. S. ilicis*, × 260. *L. S. camelliae* conidium, × 570. *M. S. ilicis* conidia, × 430.

On leaves of *Fraxinus oregana*, Manchester, Washington, W. H. Wheeler, Sept. 5, 1932. Type deposited in the National Fungus Collections.

Sections of an unopened pycnidium, as shown in FIG. 1, *C*, resemble *Phomopsis*, but the wide opening and exposed disc mark this fungus as discellaceous.

SPORONEMA HIEMALE Desm.

A specimen in the New York Botanical Gardens herbarium, Desmazières Plantes Cryptogames de France No. *81*, was studied. Examined under low magnification this species is a typical member of the Discellaceae. The pycnidia open by radiate tears, and the wall curls back, exposing the massed conidia which give the disc a reddish-tan color. The wall is rather thin, but apparently has some irregular thickenings. The conidiophores are short, 3.5–4 × 1.7 μ, and little different from the conidia. The conidia are cylindric with very truncate ends, measuring 6–8 × 2 μ. They adhere in long chains and separate with difficulty (FIG. 3, *N*).

The leaves bearing the fungus also bear black sclerotia. One of them was sectioned (FIG. 3, *O*), and it was found that *S. hiemale* was fruiting on the sclerotia as well as on the leaf. No mycelium could be seen penetrating the sclerotia from the base of the pycnidia. In fact, the two blend so gradually that they appear to be parts of one organism.

Von Hoehnel (**5**) states that no doubt *S. hiemale* is a *Schizothyrella*. However, the wall is black and such fleshiness as is present is a character of the spore-mass and not of the wall. The fungus is therefore a member of the subfamily Discellae rather than of the Patellinae, and should be placed in the genus *Sirexcipula*.

Sirexcipula hiemalis (Desm.) comb. nov.

Sporonema hiemale Desm. Ann. Sci. Nat. Bot. III. **16**: 330. 1851.

SPORONEMA ILICIS Earle.

The type material of this species, on *Ilex opaca* Ait., was studied. Of many fruiting bodies examined, only one contained conidia. The conidia are large, 12–15 × 3.5 μ, hyaline, oblong-elliptic with acute ends (FIG. 2, *M*). They are sometimes narrower in the middle, but no septa were found. The fruiting bodies in the sections show no upper wall (FIG. 2, *K*). On the evidence of these sections and the examination

of unsectioned fruiting bodies the fungus would be placed in the Melanconiaceae under *Gloeosporium*. *G. aquifolii* Penz. has conidia which answer the above description. However, two specimens labeled *"G. aquifolii"* which the writer examined are definitely not like Earle's type. The upper wall of *Sporonema* species is often poorly developed. It may be that examination of fresh material of *S. ilicis* with young fruit bodies would show upper wall tissue as was found in *S. camelliae*. More evidence is needed before *S. ilicis* is rejected.

SPORONEMA LARICINUM Sacc.

The specimen examined was No. *1709* of D. Saccardo's Mycotheca Italica, on twigs of *Larix europaea* DC. The pycnidia are dark, erumpent, and oval in section (FIG. 1, *F*). The walls are thick, 40–59 μ above and 35 μ below, and are composed of pseudoparenchymatous cells. The cavity is crowded with conidia. These were described as histogenous, but those nearest the walls can be seen to develop on tiny papillate appendages of the wall cells. The conidia are small, 4.8–5.5 $\times$ 1.5–2.5 μ, and rather angular-ovate due to crowding (FIG. 1, *B*).

S. laricinum differs from the type, *S. phacidioides*, in several respects. The pycnidial wall is thicker, always complete, and is dark at the base and sides. The pycnidia are nearly free at maturity, uniting with the host tissue only at the base, and the conidiophores are rudimentary.

SPORONEMA OXYCOCCI Shear.

Many collections of *S. oxycocci* have been examined, including one collected and identified by Shear on *Rhododendron maximum* L. This collection, which he made at Occoquan, Va., on Nov. 1, 1922, is in the National Fungus Collections. The pycnidial wall in this species is thick and dark below (FIG. 2, *G*). The upper wall tapers off in thickness from the margin to the center, where it is sometimes thin or incomplete. The conidiophores are short, about as thick as the spores, and oblong or subglobose in shape. The conidia are uniformly cylindric, usually straight, and obtuse at the ends, measuring 12–18 $\times$ 2–3 μ (FIG. 2, *H*). The length and uniformity of the conidia are striking when compared with the conidia of *S. phacidioides*, the type. *S. oxycocci* has a wide host range. In addition to the type host, *Vaccinium*, it has been found on *Rhododendron*, *Leucothoë*, *Stransvaesia*, and, as noted above, on *Camellia*.

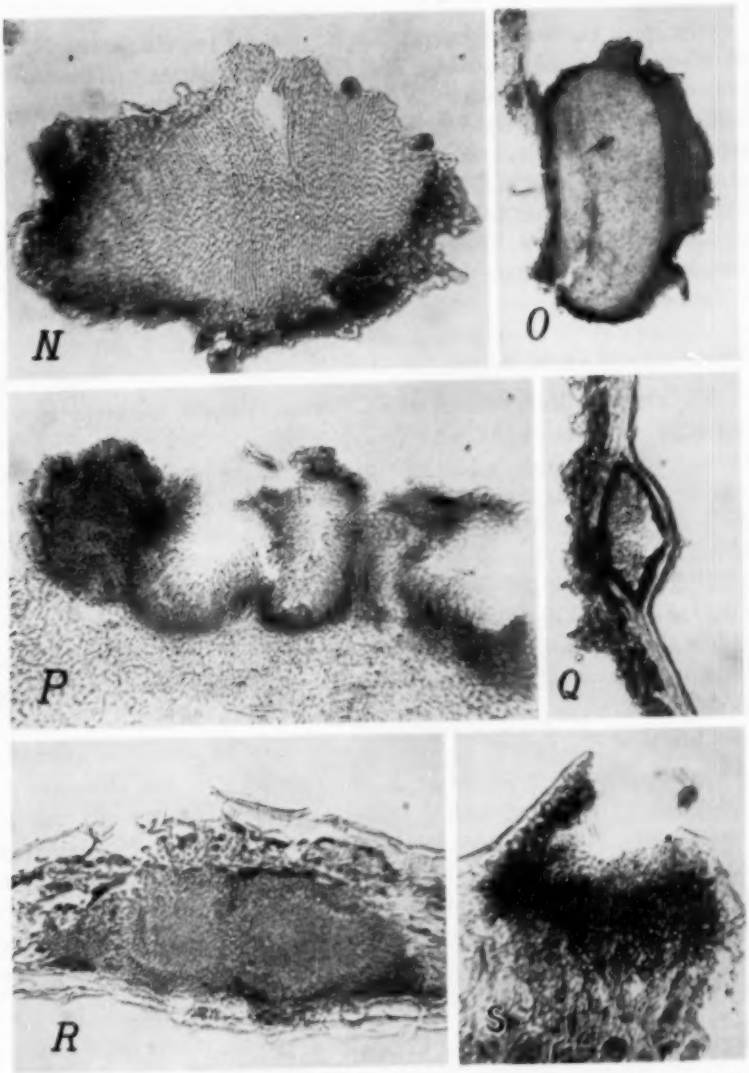

FIG. 3. *Sporonema* spp. Vertical sections of pycnidia. *N. S. hiemale*, × 260. *O. S. hiemale* pycnidium on a sectioned sclerotium, × 60. *P. S. campanulae*, × 280. *Q. S. diapensiae*, × 280. *R. S. punctiforme*, × 280. *S. S. camelliae*, × 280.

SPORONEMA PALLIDUM Ell. & Ev.

The type, Fairman's specimen No. *58*, collected at Ridgeway, N. Y., in May, 1889, shows pycnidia that are either separate or closely appressed in twos or threes, even fusing. The aggregated pycnidia are marked on the top by one or two longitudinal grooves. These grooves superficially suggest a split in the wall, but they occur where the walls of two pycnidia join, and they do not open into the cavity.

The fungus is not a *Sporonema*, as stained conidia reveal that the conidia are 1-septate. They are also catenate. If more study does not lead to its transfer from the Discellaceae, it will probably prove to be a *Siropatella*.

SPORONEMA PLATANI Bäuml.

Klebahn (**7**) demonstrated that *S. platani* is one of the many imperfect states of *Gnomonia veneta* (Sacc. & Speg.) Kleb. The specimen examined was Bäumler's No. *989*, on dead leaves of *Platanus occidentalis* L. collected at Aupark, near Posonium, Hungary. The pycnidia are black and open by splitting. The wall is usually from 18 to 24 μ in thickness and is composed of olivaceous to nearly black polygonal cells. The conidia are hyaline, continuous, oblong-ovate, 7.5–11 × 3–4.2 μ, borne on subulate conidiophores measuring 8–10 × 3.5–4 μ. A sectioned pycnidium and conidia are shown in FIG. 1, *E*. It will be noted that the pycnidium is multiocular. This character is often more distinct than in the pycnidium illustrated, as can be verified by reference to Klebahn's (**7**) figures 45–50. It may even be shown by partially fused individual pycnidia as in *S. campanulae*. In this character *S. platani* shows a morphological relationship to *S. campanulae* and *S. punctiforme*. Both of these species have been placed in *Placosphaeria* by some authors, as pointed out in the notes on those species. The writer believes that such multilocular species are not *Sporonema*. Therefore *S. platani* should be placed with the similar *S. campanulae* and *S. punctiforme* in the form genus *Placosphaeria*. The following new combination is proposed.

Placosphaeria platani (Bäuml.) comb. nov.

Sporonema platani Bäuml. Österr. Bot. Zietschr. **40**: 17–18. 1890.
Discella platani Oud. Nederland Kruidk. Arch. 2 Ser. **3**: 150. 1878.
Discella platani Peck. Ann. Rept. N. Y. State Museum **29**: 49. 1878.
Discula platani (Peck) Sacc. Sylloge Fungorum **3**: 674. 1884.
Fusicoccum veronense C. Mass. Soc. Bot. Ital. Bul. **1900**: 255. 1900.

SPORONEMA PUNCTIFORME Fckl.

Petrak (**8**) lists as synonyms *Phyllachora punctiformis* Fckl., *Placosphaeria punctiformis* Sacc., *Phyllosticta asperulae* Sacc. & Fautr., *Phyllosticta decipiens* C. Mass., and *Sporonema asperulae* Petr.

The specimen distributed by Petrak in his Mycotheca Carpatica (No. *68*) as *Sporonema asperulae* was examined, as well as *S. punctiforme* collected by Petrak (No. *438*) in Sept., 1926. They are identical, as Petrak states. The species agrees with *S. phacidioides* in conidial characters (FIG. 2, *J*), but has a very thin, complete upper wall. There is distinct evidence in sections (FIG. 3, *R*) that conidial production begins at several points in the infected tissue which merge as they enlarge. Although this character is not so conspicuous as in *S. campanulae*, it undoubtedly influenced Saccardo's decision to place the species in the genus *Placosphaeria*. The writer believes that it is morphologically closer to *Placosphaeria* than to *Sporonema*.

SPORONEMA RAMEALE Desm.

Only a few *Sporonema* pycnidia could be found on the specimen available, Desmazières Plantes Cryptogames de France, No. *82*. Externally it resembles *S. punctiforme* with a very thin membraneous upper wall. The conidia are comparatively few and large, measuring 12–20 $\times$ 4–6 μ. According to Desmazières they are 10–15 $\times$ 2.5 μ. They are borne on short, sometimes 1-septate, conidiophores which are pinched at the apex. FIG. 2, *I*, shows a portion of the base of a pycnidium with a few conidiophores and conidia silhouetted at one edge.

Von Hoehnel (**5**) states that *S. rameale* is possibly a *Phomopsis*. The membraneous nature of the upper wall, found in this specimen, does not bear this out; however, the scanty material studied does not justify an opinion.

SPORONEMA VIOLAE nom. nud.

According to the label this specimen was sent to Ellis by B. T. Galloway in Sept. 1894, and was named by Ellis and Everhart. Apparently it is unpublished. The specimen is in too fragmentary a condition to permit study.

The following species have been previously transferred to other genera with, apparently, general acceptance.

SPORONEMA GLANDICOLA Desm.

Saccardo (**10**) lists this fungus as a synonym of *Phoma glandicola* Lév. No. *83* of Desmazières Plantes Cryptogames de France was ex-

amined and sectioned. The pycnidia are thick-walled, depressed-hemispheric, or sometimes nearly conic (FIG. 1, *D*). The substratum showed many scars where pycnidia had separated from the host, but bore no remnants of the pycnidia. There was no evidence that the pycnidia open in a discellaceous manner exposing the hymenium. When conidia are present they fill the cavity. The conidial measurements vary in different pycnidia, as noted by Grove (**4**). He states that the smaller conidia (6–7 × 1.7 μ) are found in solitary pycnidia, while closely clustered pycnidia contain larger spores (10–12 × 2.5–3 μ). This explanation was not checked as the material was limited. No conidiophores were found in the sections studied. However, Grove reports finding them and that their size is 8–14 × 2.5 μ. He places *S. glandicola* in *Phomopsis* as a synonym of *P. glandicola* Grove. This is probably correct, although he reports no stylospores and the writer observed none.

SPORONEMA CASTANEA Mass., SPORONEMA DUBIUM Mass., and SPORONEMA QUERCICOLUM Mass.

Shear and Dodge (**11**) have pointed out that these species are not properly members of the genus *Sporonema*. They assigned *S. dubium* and *S. quercicolum* to *Sclerotiopsis concava* (Desm.) Shear & Dodge. *S. castaneae*, they found, is a different species, belonging either in *Sclerotiopsis* or *Pilidium*. Specimens of *S. quercicolum* and *S. castaneae* were examined. The conidia of *S. quercicolum* are identical with those of *S. concava*, while those of *S. castaneae* are larger and lunate. Both species are properly excluded from *Sporonema*. The writer has not seen *S. dubium*.

SPORONEMA PULVINATUM Shear.

The type collection of this species on *Vaccinium* leaves was studied. Shear and Dodge (**11**) listed *S. pulvinatum* as a synonym of *S. concava*, but no fungus that could be assigned to *S. concava* was found. There are some large, smooth pycnidia which superficially are like *Sclerotiopsis*. Sections proved them to be *S. oxycocci* with cylindrical conidia measuring 15–17 × 2 μ. The conidia of *S. pulvinatum* were described as 6–8 × 2–2.5 μ. The fungus originally studied on this collection has been used up or lost and, in any event, it was transferred from *Sporonema* by its author.

The writer has not seen the following species: *S. catalpae* P. Henn., *S. dubium* Mass., *S. epiphyllum* (Fr.) Shear, *S. guepini* (Mont.) Sacc., *S. nigrificans* Richinger, Baumgartner, Petrak, and Szatala, *S. obtura*

tum (Fr.) Sacc., *S. provinciale* Mont., *S. robiniae* Sacc., *S. wallrothii* Mont.

DISCUSSION

Sporonema and the other Discellaceae were set apart as a family on the basis of pycnidial characters, chiefly the manner of opening and the disc or cup-like appearance of the mature fruiting body. Study of the species shows a wide variation in other wall characters, particularly the thickness and distribution of thickness. The type, *S. phacidioides*, has a thin, nearly colorless lower wall and an upper wall which may be thick or, more often, nearly lacking. No other species studied shows the same combination of wall characters. *S. laricinum* has a thick wall both above and below, as does the new *S. fraxini*. *S. camelliae* has a wall which is thick and dark at first both above and below, but breaks up and is not found in mature specimens. *S. oxycocci* has a thick, dark lower wall which tapers out at the top until it is quite thin or even occasionally incomplete. The wall of *S. diapensiae* shows rather uniform thickness, uniformly colored. A study of the species which the writer has not seen might show that there are two or more groups which could be separated on the basis of the wall characters. There is so little uniformity in the walls of the species studied that to attribute generic significance to these wall characters would result in a new monotypic genus for nearly every species.

SUMMARY

1. Eighteen *Sporonema* species have been studied and compared.

2. The transfer of *S. hiemale* Desm. to *Sirexcipula* is proposed on the basis of its catenate conidia.

3. The transfer of *S. platani* Bäuml. to *Placosphaeria* is proposed because of the multiloculate nature of the pycnidia.

4. *Sporonema pallidum* Ell. & Ev. is shown to have septate conidia and is rejected as a *Sporonema*.

5. A new species on the leaves of *Fraxinus* is described as *S. fraxini* Limber.

6. It is proposed that *Excipula diapensiae* Rostr. be transferred to *Sporonema* to give it an acceptable name under the international rules.

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A NEW RHIZOCTONIA FROM EL SALVADOR ASSOCIATED WITH ROOT ROT OF COFFEE [1]

BOWEN S. CRANDALL AND JAIME GUISCAFRE ARILLAGA [2]

(WITH 2 FIGURES)

According to information available from coffee growers, root diseases of coffee, *Coffea arabica* L., have become a problem in El Salvador only within the past 25 years. Before the Centro Nacional de Agronomia was established, particularly from about 1938 to 1943, specialists of the Servicios Tecnicos de la Asociacion Cafetalera de El Salvador devoted considerable time to these diseases.

During this period there were various opinions as to what pathogens were responsible, but no final conclusions were reached, although the fungus *Rosellinia bunodes* (Berk. & Br.) Sacc. was generally thought the most likely suspect. Some confusion was caused by attempts to separate the diseases on the basis of symptoms alone. The most consistent observations made by the earlier workers were with regard to associated environmental conditions. It was noted that diseases regularly appeared after the shade trees, *Inga* spp., had been lost through disease, and that often they appeared after the humus layer of the soil had been lost or destroyed.

Since 1944, when the Centro was established, it has carried on the investigations on coffee root-rot as one of its most important projects. Three distinct diseases have been recognized, namely the black root-rot, here reported, a *Fusarium* root-rot with which *Rhizoctonia solani* Kuehn is associated, and a root-rot caused by *Helicobasidium compactum* Boedijn. In all three, the pathogens responsible are apparently greatly influenced by environment. All three involve *Rhizoctonias*, and all three attack the coffee plant at any age, from young plants in the nursery

[1] A contribution from the Centro Nacional de Agronomia, a technical agricultural organization, operated jointly by the governments of El Salvador and the United States. United States participation in this work was carried out as part of the program of the U. S. Operations Mission to El Salvador, administered by the Foreign Operations Administration.

[2] Respectively, Agriculturist (Research Adviser—Pathology), U.S.O.M. to Cuba and Chief Agriculturist (Director—Dept. de Asesoramiento Tecnico), U.S.O.M. to El Salvador.

to mature ones. And, in all three, evidence exists that nutrition or deficiency problems may, in part, be responsible for the susceptibility of the host to the pathogen. Progress reports on studies of these diseases have been published (**3**, **4**) in El Salvador.

Black root-rot is perhaps the most spectacular of the diseases and in general has given the accepted name for coffee root-rot (podredumbre negra). Attacked trees often die rapidly, usually with their leaves still clinging to them, although almost as often the decline may be slow enough to permit the gradual loss of leaves. A pronounced swelling is usually present above the collar, and the root below ground is covered with an incrustation of soil held to the root by exudate from the tree and fungus hyphae, regularly *R. solani*. The perfect stage of this fungus, *Pellicularia filamentosa* (Pat.) Rogers, is often produced on such trees. A different *Rhizoctonia* is found in the smaller rootlets and also in the xylem and phloem of the trunk of the tree, often to a height of a foot or more above the ground line. This *Rhizoctonia* is here being described as a new species and named for Dr. Felix Choussy, long time worker in El Salvador on coffee.

Rhizoctonia choussii sp. nov.

Mycelium in cultura rhizoctonioideum; rami laterales ad conjunctionem constricti, septo primo plerumque 25 μ e conjunctione remoto; hyphae aeriae sparsae, hyphae vegetae super medium et in eo hyalinae usque bubalinae, 6.25–9.5 μ, interdum usque 13 μ, ramis lateralibus saepe ad angulos 90° sed saepius 45–50° enatis, dein curvatis et parallelibus; cultura pallide bubalina, e pseudosporis granulosa, post horas 24 valde zonata, sporodochiis carens; pseudosporae in catenis 3–20 sporarum monilioideis ramosis dispositae, plerumque 8.3–11.2 × 16.5–19.5 μ.

In radicibus et truncis *Coffeae arabicae*, El Salvador.

Mycelium in culture *Rhizoctonia*-like. Lateral branching characteristically constricted at the point of union, with the first septum usually 25 μ from the point of junction. Aerial hyphae sparse. Vegetative hyphae on and in the media, colorless to buff, 6.25–9.5 μ, occasionally thickening to 13 μ. Lateral growth often at right angles but more commonly making a 45 to 50 degree angle with, curving, and then growing parallel to, the parent hyphae. The culture on Difco corn meal agar is regular in growth, pale buff in color and granular in appearance due to masses of pseudo-spores. Pronounced 24 hour zonation. Sporodochia absent. Pseudo-spores in long (3–20 spores), branched, monilia-like chains, mostly 8.3–11.2 × 16.5–19.5 μ. (FIG. 1, *A–F*.)

On roots, rootlets and lower portions of trunks of *Coffea arabica* L., *Crotalaria vitellina* Ker., *Desmodium nicaraguense* Benth & Oerst., and *Leucaena glauca* (L.) Benth, El Salvador. Type culture (ES-*239*)

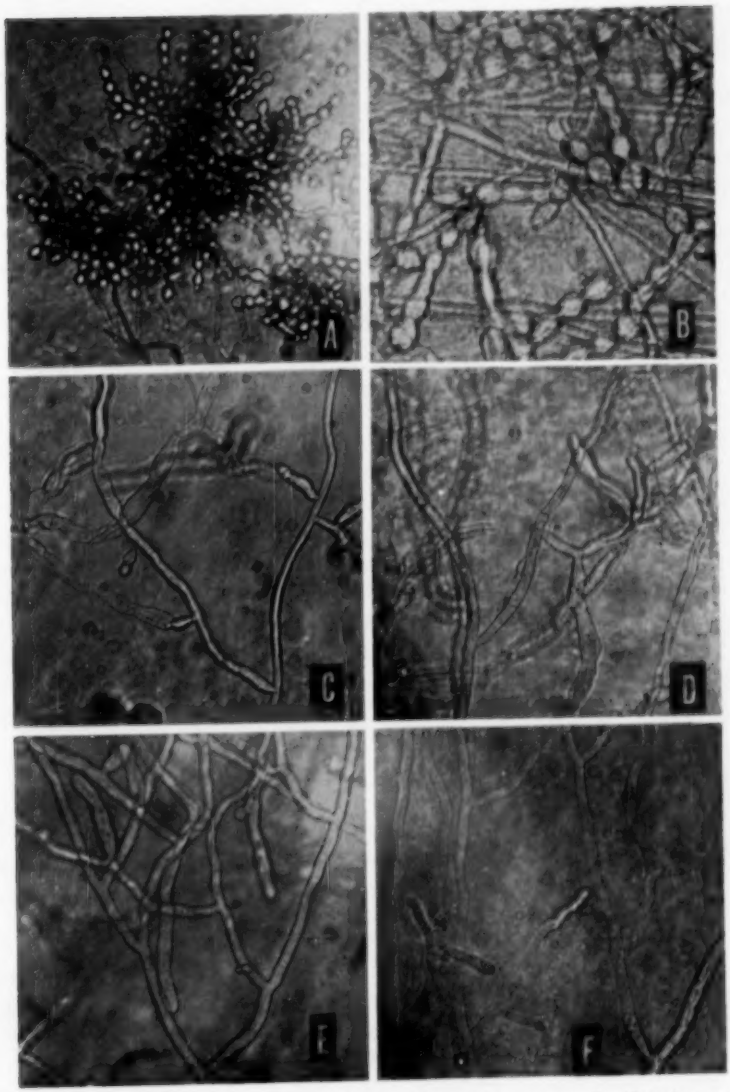

FIG. 1. *Rhizoctonia choussii*. *A*. Masses of pseudo-spores. *B*. Pseudo-spores. *C, D, E, F*. Characteristic branching and constriction of hyphae.

deposited in The National Fungus Collections, Beltsville, Maryland, the American Type Culture Collection, Washington, D. C. and the Centralbureau voor Schimmelcultures, Baarn, Holland.

R. choussii is easily distinguished from *R. solani* by its more delicate hyphae, light color, presence of pseudo-spores and absence of fern-like growth; from *R. repens* Bernard, *R. borealis* Curtis and *R. subtilis* Burgeff by the absence of sporodochia and from *R. monilioides* Curtis by the absence of aerial hyphae.

R. choussii is of the type commonly considered as mycorrhizal and it is suggested that it may well be one which, under certain environ-

FIG. 2. *A.* Sterile *Poria* sporophore on trunk and nearby soil of coffee tree with black root rot. *B.* Same excavated to show below-ground condition.

mental conditions, becomes parasitic. Mycorrhiza have been suspected on coffee trees but their presence never has been actually proved. *R. choussii* resembles such mycorrhizal Rhizoctonias as *R. borealis, R. subtilis* and others described by Curtis (**5**).

Strong presumptive evidence exists that *R. choussii* is the cause of black root-rot. It has not been possible to inoculate healthy coffee trees directly and produce the disease, but healthy trees transplanted into inoculated soil regularly contract the disease, decline and die with the

usual symptoms. Of the cover crops regularly used with coffee, planted in inoculated soil, *Crotalaria vitellina, Desmodium nicaraguense* and *Leucaena glauca* have been found to be highly susceptible. Of these, *C. vitellina* is regularly found in the coffee plantings with black root-rot.

During the surveys of coffee plantations, the sporophore (FIG. 2, *A, B*) of a sterile, white *Poria* sp. was found on one occasion closely associated with black root rot. The tissue of this tree cultured *R. choussii* and plantings from the tissue of the sporophore in agar also produced *R. choussii*. While inconclusive, this might be evidence that we are dealing with an introduced fungus of which only one sexual strain is present.

In reviewing the literature of coffee root diseases two references to what could well be this same disease have been found. Bertoni (2) in 1897 in Paraguay described a disease with similar symptoms, first seen in Brazil in 1886 and later on his own farm in 1890. He published a figure of a *Rhizoctonia* sp. considered the cause of the disease and suggested for it the name *R. subepigea* n. sp. No published description of this species has been found. Bally (1) in Java in 1931 described a disease with symptoms also similar to black root-rot. This disease was stated to have first appeared in Sumatra and then later in Java in 1928. Muller, a mycologist working with him, isolated a *Rhizoctonia* sp. of which he gave a brief description and several figures. He stated that since this was the first time a Rhizoctonia was isolated from wood it was apparently a new species. No description or name of this Rhizoctonia has apparently been published.

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APORPIUM, A POLYPOROID GENUS OF THE TREMELLACEAE

ALCIDES RIBEIRO TEIXEIRA AND DONALD P. ROGERS

(WITH 9 FIGURES)

The genus *Aporpium* Bondarzew & Singer was described for *A. canescens* (Karst.), the designated type; six other species have been more or less doubtfully referred to the group. *Aporpium* was intended as a segregate of *Poria;* the Latin diagnosis by which the name was validly published (Mycologia **36**: 67) reads: "Poriae acystidiatae, haud fibuligerae, inamyloideae, sporis cylindricis v. allantoideis." A similar description is implied by the authors' German keys earlier published in Annales Mycologici. The name undoubtedly is formed from *α*, without, and *πόρπη*, buckle, and means "without clamps."[1] Available specimens of the type species show, however, not only abundant and well developed clamp connections, but basidia with epibasidia such as characterize the Heterobasidiomycetes and hypobasidia clearly cruciate-septate, the defining characters of the Tremellaceae. *Aporpium* therefore must be included in that family.

It has been often, and appropriately, noted that in the evolution of their basidiocarps the Heterobasidiomycetes have paralleled the Homobasidiomycetes, and that many fungi of the former subclass have first been described in the latter. Thus of the Tremellaceae *Sebacina* corresponds to *Corticium* and *Peniophora, Heterochaete* to *Epithele,* and *Eichleriella* to *Stereum* of the Thelephoraceae; *Protodontia, Protohydnum,* and *Pseudohydnum* correspond respectively to *Grandinia, Oxydontia,* and *Auriscalpium* of the Hydnaceae; *Holtermannia* and *Tremellodendron* to diverse members of the Clavariaceae; and *Protomerulius* (from the description) to *Merulius.* But thus far no truly polyporoid member of the Heterobasidiomycetes has been noted.

Aporpium is based on such a species. Its pores have relatively thin dissepiments, and are therefore angular rather than round; on sloping

[1] In Mycologia **36**: 65 it is stated that a paper including this genus "was to be published in 1941 (Sovietskaya Botanika, June 1941)." The paper does not appear in that place, but what seems to be a condensed version, without generic keys or formal descriptions (except one for the genus *Merulioporia*), was published in Sov. Bot. **1943**: 29–43.

surfaces they are oblique and decurrent; but both appearances are common enough among typical porias, and there is nothing except the basidia to suggest its disposition among the Tremellaceae. Once the fungus is compared with members of the latter family, similarities are clear enough. Its hymenium often has the characteristic subresinous appearance that permits the sorting of specimens of *Eichleriella* and *Heterochaete* from among the homobasidial collections with which they are so often filed; but this resinous appearance is not diagnostic in *Poria*. Its thick-walled hyphae are characteristic of some species of *Eichleriella*, but even more of numerous porias. Under the microscope, and especially in living material, the epibasidia can be seen to be such, rather than typical subulate polypore sterigmata, more readily than the septa can be distinguished. And as in the drier species of *Sebacina* and *Eichleriella*, cruciate septation is most surely noted when the basidia are observed in end view.[2]

The only alternative assignment of this fungus is to *Protomerulius* Möll., Protobas. *129, 172, pl. 3, f. 3, 4; pl. 4, f. 36.* 1895. Of his single species, *P. brasiliensis*, Möller wrote: "Ich fand einen echten Merulius, kein Mykolog hätte auch bei sorgsamster Betrachtung mit der Lupe Bedenken getragen, ihn zu Merulius zu stellen, jede gute Diagnose von Merulius passte auf ihn. . . . Ich setze die Friessche Diagnose der Gattung Merulius zum Vergleiche hierher: Sie passt Wort für Wort zu unserem Protomerulius (Hym. Eur. S. 591). . . ." If this similarity is accepted, *Poria canescens* differs greatly from *Protomerulius*. Admittedly Möller's descriptions are suspect; his enthusiasm over the discovery of heterobasidial analogues of various homobasidiomycetes led him, as Martin has pointed out (Univ. Iowa St. Nat. Hist. **16**: 143–149. 1934), to emphasize the attributes of these homobasidiomycetes rather than of the fungi under discussion. Nevertheless, in the absence of authentic material of *Protomerulius brasiliensis* the description may be taken at face value and *Aporpium* maintained as autonomous.

APORPIUM Bond. & Sing., Ann. Myc. **39**: 50. 10 F 1941 (absque diagn. lat.); Sov. Bot. **1943**: 30. 1943 (absque diagn. lat.); Mycologia **36**: 67. 1944; Bond., Trutovye Griby 157. 1953.

Sporophore annual, resupinate, effused; pores angular, often oblique; tubes not separable, rigid, firm, long (up to 6 mm or more); hyphae partly thin-walled, with clamp connections, branching, stainable, and partly subsolid to solid; cystidia wanting; basidia cruciate-septate; spores hyaline, thin-walled, not amyloid, smooth.

[2] For bringing out septation and clamp-connections nothing seems as satisfactory as Martin's KOH-phloxine method (Mycologia **26**: 264; Univ. Iowa St. Nat. Hist. **18**(3): 9, or **19**(3): 8, 9).

Type species: *Poria canescens* Karst.

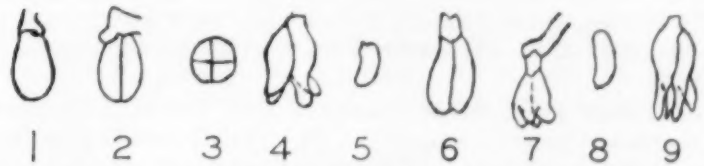
In Ann. Myc., l.c., five other binomials are listed under *Aporpium*. Since that generic name was not there validly published (Art. 44) the specific names were likewise not validly published (Art. 51 and examples), nor do they appear in the later article in Mycologia (l.c.). They then date from the 1953 publication by Bondarzew: (1) *A. rancidum* (Bres.) B. & S. ex Bond., Trutovye Griby 160. 1953, based on *Poria rancida* Bres., Fung. trid. **2**: 96. 1900, and (2) *A. vulgare* (Fr.) B. & S. ex Bond., p. 163, based on *Polyporus vulgaris* Fr., ultimis annis (Hym. Eur. 577. 1874, non Syst. Myc. **1**: 381. 1821), sensu Bres., Atti Accad. Agiati III **3**: 86. 1897, are, as exemplified by specimens from Bresadola, not heterobasidial and therefore not congeneric with *A. canescens*. (3) *A. semisupinum* (B. & C.) Bond., p. 161, based on *Polyporus semisupinus* B. & C., Grevillea **1**: 50. 1872, and including as a synonym *A. tenuiparietale* Bond. in B. & S., Ann. Myc. **39**: 50. 1941, nom. nud. (*Polyporus tenuiparietalis* Bond., Sov. Bot. **1943**: 30. 1943, nom. nud.), is, as shown by a fragment of its type, to be excluded for the same reason; no material of *A. tenuiparietale* was available for study. (4) *A. Buxi* B. & S. ex Bond., p. 161, based on *Poria Buxi* Bond., Bot. Mat. Otd. Spor. Rast. **5**: 22. 1940 (reference not seen) was likewise not available. (5) *A. Pilati* is the same as *A. canescens*. An additional species was assigned to the genus by Bondarzew: (6) *A. confusum* (Bres.) Bond., p. 164, based on *Poria confusa* Bres., Atti Accad. Agiati III **3**: 87. 1897; a specimen so determined by Bresadola is homobasidial. Thus far, then, the genus *Aporpium* must be restricted to its type species.

Aporpium Caryae (Schw.) comb. nov. FIGS. 1–9

Polyporus Caryae Schw., Tr. Am. Phil. Soc. II **4**: 159. 1832. *Polyporus argillaceus* Cke., Grevillea **7**: 1. 1878. *Poria Caryae* (Schw.) Cke., Grevillea **14**: 111. 1886; Murrill, Mycologia **12**: 82. 1920; Overholts, Mycologia **15**: 211. *f. 6, 7.* 1923. *Poria argillacea* (Cke.) Cke., Grevillea **14**: 113. 1886; Murrill, Mycologia **12**: 83. 1920; Lowe, Papers Mich. Acad. **33**: 4. 1949. *Poria canescens* Karst., Rev. Myc. **9**: 10. 1887; Meddel. Soc. Faun. Fl. Fenn. **14**: 83. 1887; Bidr. Känned. Finl. Nat. Folk **48**: 340. 1889; **62**: 84. [1903]; Bres., Atti Acad. Agiati III **3**: 79. 1897; Bourd. & Galz., Hym. Fr. 689. [1928]; Pilát, Bul. Soc. Myc. Fr. **51**: 380. [1936]; Acta Mus. Nat. Pragae **2**: 64. 1940; Atl. Champ. Eur. Fasc. 52–58: 418. *pl. 263;* p. 372, *f. 187.* 1941; Pilát & Lindtner, Glasnik, Bul. Soc. Sci. Skoplje **18**: 192. 1938; Baxter, Pap. Mich. Acad. **25**: 161. *pl. 5, f. 1, 2.* 1940; Overholts, Penn. St. Coll. Agr.

Exp. Sta. Bull. **418**: 24. 1942; Lowe, N. Y. St. Coll. For. Techn. Publ. **65**: 35. 1946. *Poria Pilati* Bourd., Bul. Soc. Myc. Fr. **48**: 230. *pl. 25.* 1932; Pilát, Acta Mus. Nat. Pragae **2**: 65. 1940; Atl. Champ. Eur. Fasc. 52–58: 408. *pl. 260;* p. 373, *f. 177.* 1941. *Aporpium canescens* (Karst.) Bond. & Sing., Ann. Myc. **39**: 50. 1941 (nom. illegitimum); Mycologia **36**: 67. 1944; Bond., Trutovye Griby 158. *f. 29, 29; pl. 34.* 1953. *Aporpium Pilati* (Bourd.) Bond. & Sing. ex Bond., Trutovye Griby 159. *f. 29, 19; pl. 33.* 1953. *Poria cordylina* Cunn., [N. Z.] Pl. Dis. Div. Bul. **72**: 23, *29. f. 17.* 1948.

Fructification coriaceous, the margin whitish, the pores warm grayish (Drab Gray[3] or lighter; or, sec. C. H. Kauffmann, Salmon-Buff to Light Ochraceous-Buff), when dry adherent, firm, Pinkish Buff through Tawny Olive to Cinnamon Brown or darkened to Clove Brown, darkest where old or bruised; margin sterile, narrow, membranous; pores nearly round when very young but soon angular, irregular, 4–6 to the mm and



FIGS. 1–7. *Aporpium Caryae.* 1–4, 6, 7, 9. Basidia. 5, 8. Basidiospores. 1–5, Indiana, *Underwood;* 6–8, *P. canescens,* type; 9, *Rogers 2213.* All × 1000.

up to 6 mm long, the dissepiments thin, at the mouths first obtuse, soon sharp, later lacerate or fimbriate, the pores often oblique and then elongate and incomplete; solid hyphae 1.5–2.3 μ, without clamps, forking, occasionally incrusted; thin-walled hyphae 1.5–2 μ in diam., with clamps throughout, short-celled and abundantly branched; cystidioles occasional, obtuse-fusiform, thin-walled, colorless and little differentiated, exceeding the basidia, about 15–18 × 4–5 μ; hypobasidia long- to short-pyriform, 7–10 × 4.5–6 μ, longitudinally cruciate-septate, often with the basal portion cut off by the diverging septa as a stalk; epibasidia tubular, terminating in a sterigma, 1.5–2.5 μ in diam., 4.5–10 μ long or sometimes almost suppressed; spores allantoid, 4.5–7 × 2–2.5(–3) μ.

On wood of both conifers and angiosperms: *Abies alba, Acer rubrum, Acer* sp., *Alnus incana, Alnus* sp., *Carya alba* (? = *tomentosa*), *Cordyline australis, Fagus grandifolia, Fagus silvatica, Fraxinus* sp., *Liriodendron Tulipifera, Liquidambar styraciflua, Nyssa sylvatica, Pinus*

[3] Capitalized color names are used in the sense of Ridgway.

Lambertiana, Populus sp., *Pyrus Malus, Tilia americana,* and numerous unidentified hardwoods.

Exsiccati: E. & E. N. Am. Fungi 2306; Calkins Florida Fungi (s.n.); Pilát Fungi Carpat. Lignic. Exs. 244.

Specimens examined:[4]

Finland: "Mustiala in *Alno incana,*" as *Physisporus canescens* Karst., *Karsten,* NY; Särkjärvi, Mustiala, XI.7.86, ad Alnum, *Karsten,* TYPE of *P. canescens,* H.

Hungary: Svidovec, prope Kosovská Polana, VII.1930, *Pilát,* TYPE of *P. Pilati,* and another specimen, BPI; Kosovaská Polana VII.1930, *Pilát,* MICH; Bílý Potok, Carpati Ross., Pilát Fung. Carpat. Lignic. Exs. 244, *Pilát,* BPI, FH, MO, NY.

Austria: Lainzer-Tiergarten bei Wien, IV.15.30, *Litschauer,* FH–B.

Ontario: woods S. of Aurora, X.26.46, *Jackson,* TRTC 21475, NY, NYSCF; Hatchley, Brant Co., X.21.46, *Cain,* TRTC 21384, NYSCF; London, XII.89, *Dearness 1343,* NY, BPI; Mt. McKay, Ft. William, Thunder Bay D., IX.4.44, *Cain,* TRTC 19912, NYSCF; Ottawa, IX.22.83, *Macoun 76–(149),* NY; VIII.21.92, *Macoun 32,* NYS.

Maine: Kittery Point, X.8.22, *Thaxter,* Weir 6, FH 3533, NY, BPI; Boarstone Camp, Piscataquis Co., IX.12–14.05, *Murrill 2517,* NY.

New Hampshire: Chocorua, IX.1908, *Farlow 47,* BPI; Hanover, Aut. '09, on *Populus, Lyman 5,* NY.

New York: Downville, X.24.48, *Lowe 3052, 3053,* NYSCF; Jamesville, XI.7.48, *Lowe 3527,* NYSCF; Newcomb, X.6.41, *Lowe 1900 & Zobel,* NY, NYSCF; Cornell Camp, Newcomb, VIII.17.32, *Eno 45,* Overholts Herb. 19855, NYSCF; North Greenbush, X.10.14, *House 14240,* NY, BPI, NYS; Pilot Knob, Washington Co., XI.4.44, *House,* NYS; Shelbourne, X.24.48, *Lowe 3516,* NYSCF; Tully, X.15.44, *Lowe 11,* NYSCF; Warrensburg, IX.24.36, *Carlberg 40,* NY, BPI; IX.4.40, *Lowe 1751,* BPI, NYSCF; IX.7.42, *Lowe 2432,* NYSCF, MICH; VIII.7.42, *Lowe 2028,* NYSCF; and VIII.12.42, *Lowe 2094,* NYSCF; X.20.35, *Lowe 1620,* NYS.

[4] From these herbaria: BPI, National Fungus Collections, Beltsville; BPI–L, their Lloyd collection; FH, Farlow Herbarium; FH–B, its Burt collection; H, Botanical Museum, Helsinki; K, Royal Botanic Gardens, Kew; MICH, University of Michigan; MO, Missouri Botanical Garden; NY, New York Botanical Garden; NYS, New York State Museum; NYSCF, New York State College of Forestry, Botany Department; PDD, New Zealand Plant Diseases Division; PH, Philadelphia Academy of Sciences; S, Naturhistoriska Riksmuseum, Stockholm; TRTC, University of Toronto, Cryptogamic Herbarium.

New Jersey: Hopewell, X.27.45, *Lowe 3072,* NYSCF.

Pennsylvania: Nazareth, *Schweinitz,* and in Hickory, *Schweinitz* (two packets, but not surely two collections), TYPE OR PARATYPES of *P. Caryae,* PH; *Schweinitz,* ISOTYPE, NY, BPI; near Philadelphia, XI.1885, *Gentry,* NY.

Maryland: Hinson's Run, X.21.24, *Weir,* BPI.

District of Columbia: Washington, IX.1921, *Weir 20236, 21548;* and *Gravatt;* all BPI.

Virginia: Great Falls, IX.23.21, *Weir 20046,* BPI; Richmond, X.23.45, *Lowe 3027,* NYSCF.

North Carolina: Chapel Hill, III.19.22, *Grant 5011;* and IV.18.20, *Couch 4270;* both BPI.

Florida: XII.1885, *Calkins 56,* NY; *Calkins,* Florida Fungi (s.n.) as *Polyporus cinereus,* NY; near Jacksonville, winter of 1887, *Calkins,* in E. & E. N. Am. Fungi 2306, NY, BPI, BPI-L; Rochelle Hammock, near Gainesville, IX.3.54, *A. M. & D. P. Rogers 2313,* NY; Magnesia Springs, IX.3.34, *Rogers 2212,* NY.

Michigan: Ann Arbor, XI.1913, *Kauffman 3415,* BPI, *Kauffman 739,* MICH, *Kauffman* s.n. (3 collections), MICH; VII.1.15, *Kauffman* s.n., MICH; and VII.27.11, *Kauffman 3394,* BPI; Neebish, IX.1907, *Harper,* F526128, BPI.

Ohio: *Morgan 329,* NY; *Morgan,* BPI; *Lloyd 3561,* NY; Cincinnati, *Morgan,* BPI-L 43600; Linwood, VIII.18.20, *Lloyd 22257,* BPI-L; and IX.22.20, *Lloyd 44613,* BPI-L; Miami Valley, *Morgan,* NY.

Indiana: Fern, Putnam Co., XII.84, *Underwood,* NY; Scottsburg, IX.1907, *Weir 20263,* BPI.

West Virginia: Marlinton, VII.30.24, *Baxter III,* BPI.

Kentucky: Crittenden, X.5.14, *Lloyd 17011,* BPI-L.

Louisiana: "ex Ellis"; *Langlois 178;* I.9.94, *Langlois* "= *2792*"; X.18.98, *Langlois 2762;* I.21.89, *Langlois 1609;* XII.20.86, *Langlois 973;* VII.27.89, *Langlois 2030;* Grand Bois, XI.16.97, *Langlois 2552,* all BPI; *Langlois,* Lloyd herb. 3561, NY; St. Martinville, VII.22.89, *Langlois 1881,* BPI, X.3.97, *Langlois 2553,* NY.

Wisconsin: Devil's Lake, X.20.24, *Baxter IX,* BPI.

Illinois: River Forest, VII.1909, *Harper,* F523148, BPI.

Kansas: *Cragin 193,* NY.

Washington: Elwha R., VII.12.39, *Smith 14291,* MICH; Newport, 1918, *Weir 12517,* BPI.

California: Colfax, *Harkness 958,* LECTOTYPE of *P. argillaceus,* NY, K, BPI; Sierra Nevada, *Harkness 1000,* SYNTYPE, BPI, K.

Venezuela: *Fendler,* comm. M. A. Curtis, SYNTYPE of *P. Fendzleri,* NY.

New Zealand: Claudelands Res., Auckland Distr., XI.1946, *Cunningham 5428*, TYPE of *Poria cordylina*, PDD, NY.

Further distribution: reported also from Siberia, Bulgaria, Jugoslavia, and France, and in the western hemisphere from Yukon Territory, Vermont, Connecticut, and Georgia.

Specimens of *A. Caryae* may be filed in various herbaria under the synonyms here listed, and also under the names *Poria cinerea* (Schw.), *Poria Fendzleri* (B. & C.), and *Poria gilvescens* Bres. The type of *P. cinerea* has not been reexamined and its characters are unknown. If it proves to belong here, the name *A. Caryae*, already established by Murrill's, Overholts's, and the present authors' restudy of the type, is to be preferred. The lectotype of *P. Fendzleri* (B. & C.), Cuba, *Wright 389*, in herb. Berkeley (K) and in NY, is quite a different thing, with fusiform, abruptly apiculate cystidioles 4–7 μ in diameter; no mature basidia were seen. *P. gilvescens*, as Dr. J. L. Lowe has recently reported (in litt.), must also be quite a different fungus; as represented by two of the original specimens (Hungary: Prenčow, M[onte] Sytno, VII.19.89, in ligno putrido Fagi sylv., *Kmet*, S; France: Ad trabem quercinam, *Bourdot 3810*, BPI), it has clavate-cylindric basidia and narrow-allantoid spores; but the two specimens represent two different species; the latter has relatively thin-walled, granule-incrusted hyphae, and the former thick-walled hyphae frequently septate and sometimes bearing conspicuous clamp-connections; most American and some European material under that name is the present species. Since Overholts recognized (Penn. St. Coll. Agr. Exp. Sta. Bull. **418**: 46. 1942) that *P. gilvescens* Bres. 1908 sensu Overh. has the earlier synonym *P. Caryae* (Schw. 1832) Cke. 1886, it is difficult to understand how the Bresadola name ever was taken up for this species. It should be further noted that although Pilát (Atlas, l.c.) describes the spores of *P. canescens* as ovate, they are allantoid in his exsiccatum (*244*). The type of *P. Pilati* also has cruciate-septate basidia, allantoid spores, and clamp-connections on the thin-walled hyphae. It is a dark, apparently over-mature specimen, with the pores much torn in drying, well illustrated by the photographs published by Bourdot and by Pilát and matched by some American material; but a minute area of renewed growth on the portion examined shows the more usual appearance of *A. Caryae*. The type of *P. cordylina* is also much darkened and more resinous in appearance than most material; it shows excellently the microscopic characters of the present species. The two packets of *P. Caryae* in the Schweinitz herbarium, one labeled "in hickory" in Schweinitz's hand, and one relabeled

"Nazareth" by Michener, contain the same fungus and possibly are parts of the same collection. The transfer of *Poria canescens* to the genus *Physisporus* was indicated by Karsten in 1903 (l.c.) but no binomial was published (Art. 42, par. 3) there or, apparently, elsewhere.

The writers are grateful to Mr. J. A. Stevenson and Dr. W. W. Diehl of the National Fungus Collections, Maila M. Lamb of the Farlow Herbarium, Dr. R. L. Gilbertson and Dr. J. L. Lowe of the New York State College of Forestry, Dr. H. Roivainen of Helsinki, Dr. Bessie B. Kanouse of the University of Michigan, Dr. E. D. Rudolph of the Missouri Botanical Garden, Sir Edward Salisbury and Dr. R. W. G. Dennis of Kew, Dr. G. Haglund of the Riksmuseum, Stockholm, Dr. C. E. Smith of the Philadelphia Academy of Sciences, Mr. Stanley J. Smith of the New York State Museum, and Dr. G. H. Cunningham of the New Zealand Plant Diseases Division for the loan of specimens.

It should be recorded here that Dr. Ruth Macrae of Ottawa noted independently, and earlier than the authors of this paper, the tremellaceous nature of the fungus under discussion, and has carried out a detailed study of it; her results are to be published elsewhere.

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A NEW SPECIES OF CHAETOCERATOSTOMA

W. M. Farrow

(WITH 5 FIGURES)

During recent studies of soil fungi a previously undescribed ascomycete was isolated frequently from several soil samples collected in Barro Colorado Island, Panamá Canal Zone, and Puerto Armuelles, Chiriquí Province, Panamá. The soil samples were a part of collections made in this region in July and August, 1952, by G. W. Martin and A. L. Welden under a National Science Foundation Grant.

This fungus could conceivably fall in the Chaetomiaceae, Melanosporaceae or the Ophiostomataceae. The genera in these families have several characteristics in common, notably, the presence of appendages, short or extended necks, ostioles and asci which are evanescent.

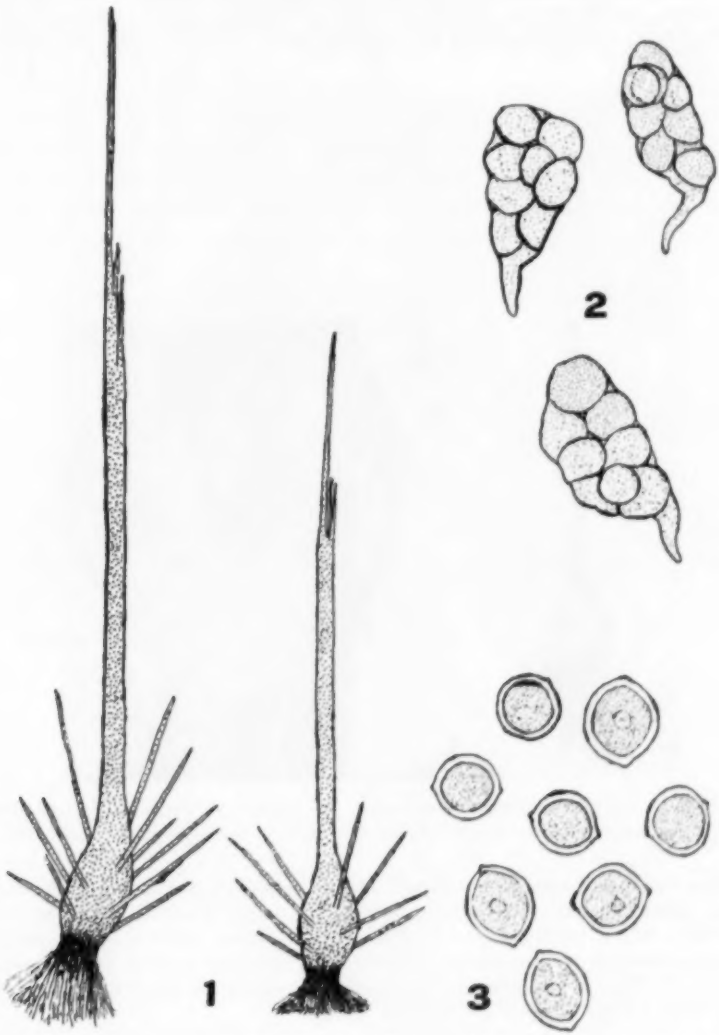
In the Chaetomiaceae the organism resembles more closely *Lophotrichus* in the membranous perithecia and spore characteristics. It differs mainly from *Lophotrichus* in the presence of spiny, lateral setae on the perithecium, in the absence of terminal hairs, and in the long, irregular length of the neck filaments. Also, in longitudinal sections (Fig. 5), there appears to be quite consistently a short stem-like structure at the base of the perithecium. In view of these differences the organism was not placed in *Lophotrichus*.

The fungus would apparently not fall in the Melanosporaceae because the spores show little variation in shape and the asci originate in a tuft at the base of the perithecium (Fig. 5) rather than forming a wall layer.

In the Ophiostomataceae the asci show an irregular distribution throughout the centrum. This resembles the condition found in the new organism, although the arrangement of the asci does not seem to be very irregular. Other similarities are in the long beaks, appendages, dark-colored perithecia and evanescent asci. One difference is found in the color of the ascospores, which are dark in the new fungus and hyaline in the Ophiostomataceae. This characteristic should not warrant excluding the organism from this family.

Dr. Julian H. Miller, who has kindly examined a culture of the fungus, agreed that the nearest form would be *Chaetoceratostoma hispidum* Turconi and Maffei (Att. Ist. Pav. **2**: 15: 144. 1918). However, it differs mainly from that species in the possession of membranous

rather than semi-carbonous perithecia, ovoid rather than spherical perithecia and subglobose as contrasted with cubical ascospores. Both, however, possess perithecia with long necks, numerous rigid setae, asci which dissolve early and dark brown spores. Based on these major

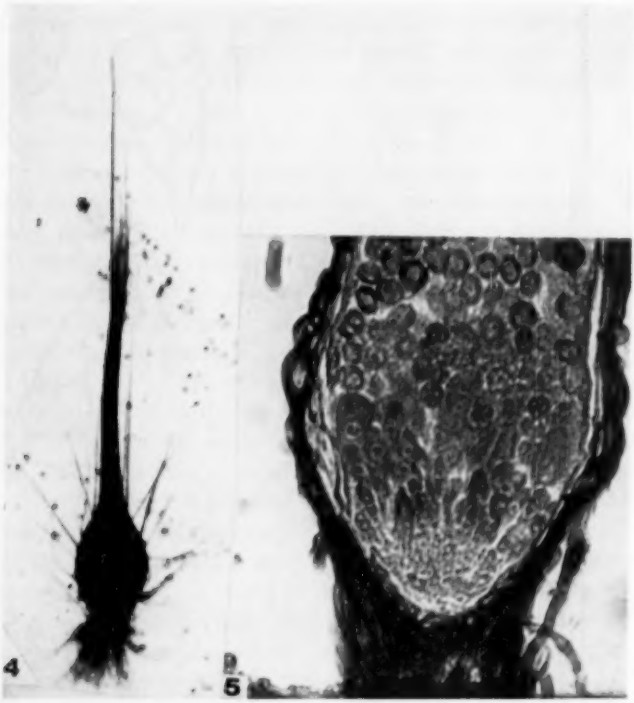


FIGS. 1–3. *Chaetoceratostoma longirostre*. Drawn with the aid of a camera lucida. 1. Perithecia, × 100. 2. Asci, × 750. 3. Ascospores, × 750.

characteristics it is proposed to place the fungus in *Chaetoceratostoma* but to describe it as a new species.

Chaetoceratostoma longirostre sp. nov.

Mycelio brachiato; hyphis 2.0–7.0 μ diam.; peritheciis brunneis, superficialibus, obovoideis, membranaceis, 148–205 × 74–118 μ, rhizoideis maturis substrato connexis; rostris praelongis, erectis vel ad basim leniter incurvis, cylindraceis, 1,012–



FIGS. 4–5. *Chaetoceratostoma longirostre*. 4. Mature perithecium, × about 100. 5. Longitudinal section of perithecium showing asci arrangement, × about 600.

2,080 μ longis, ad basim 34–36 μ diam.; ad apicem 5–17 μ; rostrorum paraphysibus longitudinis irregularis frequenter apice separatis; setulis brunneis, rigidis, septatis, 220–625 μ longis, substrato 3.5–5.2 μ diam., mox evanidis, apice rotundatis, erectis et leniter incurvis; ascis octosporis, clavatis vel cylindraceis, evanidis, aparaphysatis, 24–43 × 12–19 μ; ascosporibus immaturis hyalinis, maturitate brunneis, subglobosis, apiculatis, 8.9–12.0 × 8.5–10.2 μ; conidiis ignotis.

Mycelium branched, hyphae 2.0–7.0 μ diam.; perithecia dark brown, superficial, ovoid, membranous, 148–205 × 74–118 μ, attached to sub-

stratum with well-developed rhizoids; beaks prominent, straight or slightly curved at the base, cylindric, 34–36 μ diam. at base to 5–17 at tip and 1,012–2,080 μ tall; beak filaments frequently separating at tip and of irregular length; setae brown, rigid, septate, 220–625 μ long, 3.5–5.2 μ diam. at base and tapering to the rounded end, straight or slightly incurved; asci octosporous, clavate to cylindrical, evanescent, 24–43 $\times$ 12–19 μ; paraphyses lacking; ascospores hyaline when young, changing to dark brown at maturity, sub-globose, apiculate, 8.9–12.0 $\times$ 8.5–10.2 μ; conidia unknown.

In soil, Barro Colorado Island, Rio Sardinilla, Panamá Canal Zone, and Puerto Armuelles, Prov. Chiriqui, Panamá. TYPE: GWM *8875*, from soil collected on Barro Colorado Island in 1952. Type cultures preserved in herbarium State Univ. Iowa and distributed to National Type Culture Collection and the Centraalbureau voor Schimmelcultures, Baarn.

This is the species reported as *Chaetoceratostoma* sp. in MYCOLOGIA **46**: 639. 1954.

Abundant, mature perithecia develop in six to nine days on oatmeal flakes, cornstalks or grass stems in plain agar. Young asci in various stages of development were observed in perithecia which were grown on grass stems in plain agar, harvested the eighth day and fixed with FPA.

The writer wishes to thank Dr. G. W. Martin for helpful suggestions in preparation of the manuscript, Dr. Donald P. Rogers for assistance with the Latin diagnosis, and Mr. C. R. Benjamin for preparation of slide material.

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NOTES AND BRIEF ARTICLES

A NOTE ON THE ISOLATION OF MICROORGANISMS FROM NATURAL SOURCES

Many laboratories are engaged in the large scale isolation of representatives of fungi and the genus *Streptomyces*. In such work, one is faced constantly with the problem of eliminating undesired organisms that interfere with isolation of the ones desired. This is particularly so when one desires to isolate members of a natural population that are present in relatively small numbers.

One obvious method of eliminating undesired components of a complex mixed population and thus creating a selective environment for the desired ones is by the addition of antibiotics to the plating media. Talks with various investigators over the past year or so have revealed that this procedure may not be in extensive use, although antibiotics have been used in isolations from more specific mixed microfloras.[1] Hence, this note is to present some of our experiences with the use of antibiotic mixtures in isolating *Streptomyces* cultures as well as a broad spectrum of fungi.

The antibiotics are weighed and dissolved together in distilled water. The solution is then sterilized by passage through a sterile sintered glass filter. One ml of this solution is added to sterile plates, followed by 1 ml of the desired dilution of the material from which isolates are to be made and 20 ml of cooled nutrient agar. The whole is mixed thoroughly. After solidifying, the plates are incubated at the desired temperature until growth of the organisms occurs. They then may be isolated by the routine procedures.

By use of antibiotics combinations, soils and homogenates of material, such as rotten wood and animal dungs, can be plated at low dilutions. In practice, 1 gram of soil is added to 100 ml of water, shaken thoroughly and 1 ml pipetted into plates with the antibiotics mixture and 20 ml of agar medium. In addition, pieces of animal dung or rotten woods are homogenized for a few seconds in 50 or 100 ml of sterile water in a Waring blendor and 1 ml of the homogenate similarly plated. Quite good results have been obtained.

[1] For references see Beech, F. W., and J. G. Carr. 1955. A survey of inhibitory compounds for the separation of yeasts and bacteria in apple juices and ciders. Jour. Gen. Microbiol. **12**: 85–94.

For isolation of *Streptomyces* cultures, the following combination of antibiotics has been used: actidione 20 u./ml, polymyxin 20 u./ml, subtilin 20 u./ml, and penicillin 2.5 u./ml. The potencies noted are the final concentrations per ml of agar medium. We arrived at this combination of antibiotics by testing each singly and in various combinations against unknown soil populations and against prepared mixtures of *Streptomyces* spores. Inhibition of bacteria and not of *Streptomyces* may present more of a problem than is commonly realized. *Streptomyces* were inhibited by penicillin in the system used here and one must be careful of the level of this antibiotic that is employed. Some of the *Streptomyces* antibiotics, e.g., streptomycin, also eliminated numbers of actinomycetes from soil platings.

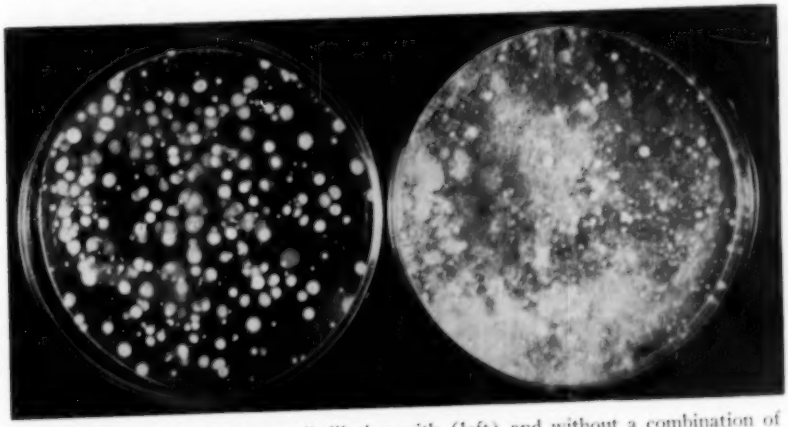


FIG. 1. Results of plating a soil dilution with (left) and without a combination of antibiotics (right).

Bacteria can be eliminated to a great extent by use of the above antibiotics combination. Some fungi present a problem, however. Various members of the Mucorales and cultures of *Trichoderma*, for example, frequently overgrow plates, rendering isolation from them quite difficult. The acitidione is not sufficient to suppress these fungi. Actidione has been used at higher levels than 20 u./ml with no marked effect of the *Streptomyces* population. There was, however, no marked decrease in the numbers of fungi. Perhaps a combination of antifungal antibiotics would solve this problem but this has not been investigated extensively. Agents that suppress radial growth of fungi might also be the answer.

The results of plating a dilution of soil with and without the antibiotics combination are shown in FIG. 1.

Even more striking results can be obtained in isolating fungi where both actinomycetes and bacteria are to be eliminated. The following antibiotics combination has given excellent results: penicillin 5 u./ml, streptomycin 10 u./ml, polymyxin 40 γ/ml, bacitracin 20 γ/ml, and aureomycin 20 u./ml. These are the final concentrations per ml of agar. The levels of polymyxin and bacitracin are not too critical. Different lots of varying potencies have been used. In practice we have used each impure material on the above weight basis with equally good results.

Use of this antibiotics combination in platings of natural materials yields plates on which bacteria or actinomycetes seldom occur. Here again, however, rapidly spreading fungi pose a problem. This problem has been solved in part by adding sodium desoxycholate to the agar medium before sterilization.[2] The final concentration in the agar is 0.03%. Fungi grow and sporulate in the presence of this concentration of sodium desoxycholate but radial growth is markedly inhibited. They grow "normally" on the first transfer to fresh medium. An extremely broad spectrum of fungi has been isolated by this method.

Whereas good success has been attained through the use of the above antibiotic combinations, better ones might well be developed especially for isolation from more specific microfloral mixtures. It appears, however, that the most efficient method is to use a combination of antibiotics, rather than to rely on one or two broad spectrum ones.—EUGENE L. DULANEY, A. H. LARSEN AND E. O. STAPLEY, Research Laboratories, Merck & Co., Inc., Rahway, N. J.

THE STATUS OF THE NAME ERYSIPHE CICHORACEARUM DC.

During a study of the host specialization of the powdery mildews from zinnia, phlox, cucurbits and several other species of hosts, it was necessary to establish the correct name and citation for the pathogen involved. According to Salmon's treatment of the group (Memoirs Torrey Bot. Club **9**: 1–292. 1900) the species is *Erysiphe cichoracearum* DC. In this monographic work Salmon did not follow the International Rules of Botanical Nomenclature.

In an effort to modernize the nomenclature of the powdery mildews according to the Rules adopted by the 1951 International Congress as they affect this group, W. B. Cooke (Mycologia **44**: 570–574. 1952)

[2] Tatum, E. L., R. W. Barratt and V. M. Cutter, Jr. 1949. Chemical induction of colonial paramorphs in *Neurospora* and *Syncephalastrum*. Science **109**: 505–511.

has pointed out that the name *Erysiphe cichoracearum* DC. (A. P. de Candolle. Flore française **2**: 272. 1805) is pre-Friesian and therefore has no standing. He suggested that the valid name for *E. cichoracearum sensu* Salmon should be *Erysiphe communis* Wallr. ex Fr. This Friesian name included mildews on several families of flowering plants. According to Salmon it included mildews of the following species: *Sphaerotheca humuli* (DC.) Burr., and var. *fuliginea* (Schlecht.) Salmon; *Microsphaera astragali* (DC.) Trev.; *Erysiphe polygoni* (DC.) Salm.; *E. cichoracearum* DC.; *E. galeopsidis* DC.; and *E. graminis* DC. According to the species of hosts listed, *Erysiphe communis* of Fries was primarily *E. polygoni*. For this reason the writer does not agree with Cooke that the valid name for *Erysiphe cichoracearum* of Salmon is *E. communis* Wallr. ex Fries. This position, however, leaves the species as treated by Salmon without a Friesian name.

Fries's reduction of the de Candolle name as a synonym of *E. communis* is a taxonomic judgment and as such is subject to review. Two questions may be raised: Is *E. communis* referable to a definite species and what is the type for the name *E. communis?* The last question can be approached only by considering on what the name was based and what has been included in the species or excluded from it in subsequent monographs.

The basonym of *Erysiphe communis* (Wallr.) Fr. is *Alphitomorpha communis* Wallr. (Verh. Ges. Naturf. Fr. Berlin **1**: 31. 1819). Under this species Wallroth included eight "Abarten," of which α is *Leguminosarum* (equals *E. pisi* DC.) and ε is *Cichoracearum* (DC.). Concerning the latter he says (p. 33) "*Cichorac. DeCandolle* beobachtete diese Art auf *Scorzonera hispanica, Tragopogon* und *Sonchus*. Ich sah nie Früchte und kann daher nicht genau bestimmen, ob sie meiner *A. communis* wirklich angehöre." This then is not the type of Wallroth's name. In Systema Mycologicum **3**: 239 there is nothing to indicate what Fries considered the typical component. Fries's note in Summ. Veg. Scand. (p. 405, last sentence of the second footnote) likewise sheds no light on this typification.

Two points should be mentioned concerning monographs subsequent to the Systema. On p. 175 of Salmon's monograph he makes *A. communis* Wallr. "(Excl. var. *gramineorum, labiatarum, cichoracearum*)" a synonym of *Erysiphe polygoni*. On p. 194 Salmon makes *A. communis* var. *cichoracearum* a synonym of *E. cichoracearum*. Although he did not typify *E. communis* he did narrow the choices from which the type could be selected. Salmon and Wallroth agree in concluding that *E. communis* in the broad sense is not typified by *cichoracearum*.

According to Salmon the first post-starting-point treatment of *communis* is as *Erysibe communis* in Ficinus and Schubert, Fl. Geg. Dresden **2**: 304. 1823, where *Leguminosarum* Wallr. is the α variety and *cichoracearum* is not treated at all. Their selection of the α, or typical, variety should be given some consideration in the typification of *Erysiphe communis*.

Schlechtendal (Fl. Berolinensis **2**: 168. 1824) recognized *Erysibe communis* (Wallr.) with *Alphitomorpha communis* as the basonym. As hosts for this species he listed *Pisum sativum, Thalictrum flavum, Polygonum avicularis, Convolvulus arvensis, Circaea lutetiana, Cynoglossum officinalis* and *Symphytum officinalis*. Of these seven species the first five are considered as hosts for *Erysiphe polygoni* DC. as treated by Salmon.

It would seem from the above evidence that *Erysiphe cichoracearum* and *E. communis* have different types. These two species can be synonymous only under a species concept so broad that it would undoubtedly be rejected by all. It further seems that *E. communis* is to be applied to a species including Wallroth's var. *leguminosarum*.

In Mérat's *Nouvelle flore des environs de Paris*, 2 ed., vol. 1 (1821), the name *Erysiphe cichoracearum* DC. is used (p. 132). Rogers (Mycologia **43**: 376–378. 1951) has established that Mérat's work was published about June, 1821. Thus Mérat's is the first post-Friesian publication, since the latest International Rules of Botanical Nomenclature states, in Article 23F: 1) that the starting point for the *Fungi cateri* is January 1, 1821; 2) that volume 1 of the Systema Mycologicum is taken as published on that date; 3) that the Elenchus is considered a part of the Systema; and 4) that other names published after January 1, 1821, do not interfere with names used by Fries in the Systema.

The evidence presented herein is sufficient to show that the name *Erysiphe communis* is not applicable to what Salmon and most others concerned with the powdery mildews call *Erysiphe cichoracearum*. Furthermore Mérat's use of *Erysiphe cichoracearum* DC. validates the name for this species. It should be cited as *E. cichoracearum* DC. ex Mérat. —JOHN A. SCHMITT, JR., University of Mississippi, University, Mississippi.

A METHOD FOR TESTING PARAFFIN UTILIZATION BY MICROORGANISMS

The utilization of paraffin and other hydrocarbons by microorganisms, particularly actinomycetes, can be used as a criterion for specia-

tion. In the genus *Nocardia*, for example, species which use paraffin as a sole carbon source are also partially acid-fast, non-proteolytic (for gelatin) and do not hydrolyze starch.[1]

The method generally used for testing paraffin utilization has been to add either melted or solid paraffin to a suitable mineral medium, inoculate, and to judge utilization or refusal by visible growth. Söhngen [2] tested paraffin utilization by adding 1% paraffin to 100 ml liquid medium in a 450 ml Erlenmeyer flask which was then heated to melt the paraffin. The paraffin was finely divided by shaking while holding the flask under the water tap for rapid cooling. Jensen [3] added "flakes of paraffin wax" to a mineral solution. Umbreit [1] put a drop of melted commercial paraffin into each tube of liquid Czapek's medium. In recent experiments the author [4] tested paraffin utilization by *Nocardia* species as follows: 50 mg of paraffin were placed into each tube of mineral medium before autoclaving. This method was unsatisfactory, since growth of *Nocardia* species on paraffin was always slow, and it was often difficult, particularly with non-chromogenic strains, to be sure of results. This necessitated repeated trials for doubtful species.

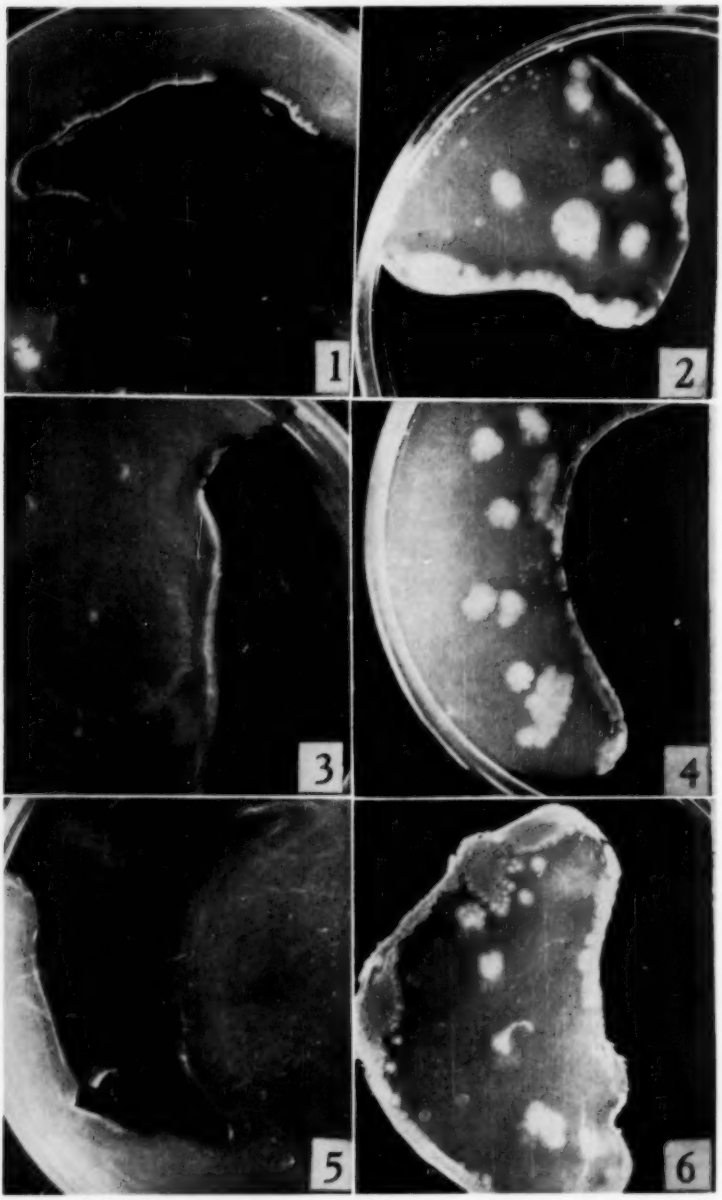
A method for testing paraffin utilization has been devised which gave unequivocal results for the thirty-seven strains of *Nocardia* tested. A description of this method follows: The bottom of clean sterile petri dishes was coated with sterile melted paraffin wax (tissuemat, Fisher Scientific Co., mp. 56–58.5° C). The coating should be thin, although the amount of paraffin is not critical. The paraffin was allowed to harden, then 10 ml of a mineral medium consisting of $NaNO_3$, 2.0 g; K_2HPO_4 (anhydrous), 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $Fe_2(SO_4)_3$, 10.0 mg; $MnCl_2 \cdot 4H_2O$, 8.0 mg; $ZnSO_4 \cdot H_2O$, 2.0 mg; agar, 20 g; distilled water to make 1,000 ml (pH was adjusted to 7.2 before sterilization) was melted and cooled to 50° C, then poured over the paraffin. When the medium was poured at about this temperature onto the paraffin, a thin layer of paraffin covered the medium, which formed an island in the bottom of the dish. The medium was then inoculated at the juncture of the island of mineral medium and the paraffin. Points on the surface

[1] Umbreit, W. W. 1939. Studies on the proactinomyces. Jour. Bact. **38**: 73–89.

[2] Söhngen, N. L. 1913. Benzin, Petroleum, Paraffinöl und Paraffin als Kohlenstoff- und Energiequelle für Mikroben. Zentralbl. f. Bakt. II. **37**: 595–609.

[3] Jensen, H. L. 1931. Contributions to our knowledge of Actinomycetales II. Proc. Linn. Soc. N. S. Wales **56**: 345–370.

[4] McClung, N. M. 1954. The utilization of carbon compounds by *Nocardia* species. Jour. Bact. **68**: 231–236.



FIGS. 1–6.

of the agar medium may also be inoculated. Controls of mineral agar without paraffin were similarly inoculated at the same time. The cultures were then incubated for three weeks at 28° C in a saturated atmosphere. Results were read by comparing the growth on the control plates with those containing paraffin. Although some strains of *Nocardia* grow sparsely on the mineral medium alone (FIG. 1), the growth on paraffin of strains which use it as an energy source is much more luxuriant (FIG. 2).

This method is also suitable for testing paraffin utilization by bacteria and other fungi.—NORVEL M. MCCLUNG, Department of Botany, University of Kansas, Lawrence, Kansas.

A NEW SPECIES OF AMANITA FROM FLORIDA

Amanita neglecta sp. nov.

Pileo convexo-expanso, 4–4.5 cm lato, albo, verrucis albis obtecto, grato; lamellis adnatis, confertis, albis, 7 mm latis; sporis oblongo-ellipsoideis, levibus, 6–7 × 3–4 μ; stipite glabro, albo, 4–4.5 × 1–1.5 cm; annulo supero, amplo, albo, reflexo; volva adnata, alba, evanida.

Pileus convex to expanded, gregarious, 4–4.5 cm broad; surface white, ornamented with many white raised volval patches, more pyramidal on the disk as in *A. aspera*, margin even, entire; context 2 mm thick, white, unchanging, odorless, mild; lamellae adnate, crowded, white, unchanging, 7 mm broad; spores oblong-ellipsoid, smooth, about 6–7 × 3–4 μ; cystidia none; stipe tapering upward, smooth, glabrous, white, unchanging, 4–4.5 × 1–1.5 cm; annulus superior, skirt-like, persistent, white; bulb small, ovoid, white, the volva limb entirely friable at an early stage.

Type collected by W. A. Murrill on exposed deep humus in Gainesville, Fla., Sep. 16, 1950 (*F 32887*). Known only from the type locality, where it appeared at intervals for some time. In my nomenclature the species would be assigned to *Venenarius*.—W. A. MURRILL.

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1. Stock number 12, unidentified *Nocardia* sp. from oil sands. Mineral medium without carbon source. 2. Stock number 12, mineral medium plus paraffin. 3. *Proactinomyces ruber* (Casabó) Bald. Mineral medium without carbon source. 4. *P. ruber*. Mineral medium plus paraffin. 5. Stock number 42, unidentified *Nocardia* sp. from soil. Mineral medium without a carbon source. 6. Stock number 42, mineral medium plus paraffin.

The incubation period was 3 weeks for all cultures. All figures slightly reduced. The pictures were made after melting the paraffin on a hot plate.

THE TUBERALES [1]

A fructification of Dr. Gilkey's continuing studies of the Tuberales is this most recent number of the North American Flora. Of the 64 species now recognized from the continent, five are here first described; they were found in Illinois, Michigan, New Mexico, Washington, and California. Three of these fall in two new genera, *Carbomyces* and *Densocarpa;* and the earlier name *Lespiaultinia* has replaced the often employed but illegitimate *Delastreopsis.* Users of the work will wish to note that the author's paper therein cited as "Mycologia **47** (in press)" appeared early, in Mycologia **46**: 783–793. 1954.

Because of anticipated difficulty in fitting further contributions to the N.A.F. into the scheme of orders and volumes originally laid out for that work, with this number is begun a second series, in which parts will be numbered in order of issue.—D. P. R.

[1] GILKEY, HELEN M. Tuberales. North American Flora II. **1**: 1–36. 1954.—The New York Botanical Garden, $1.60.

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